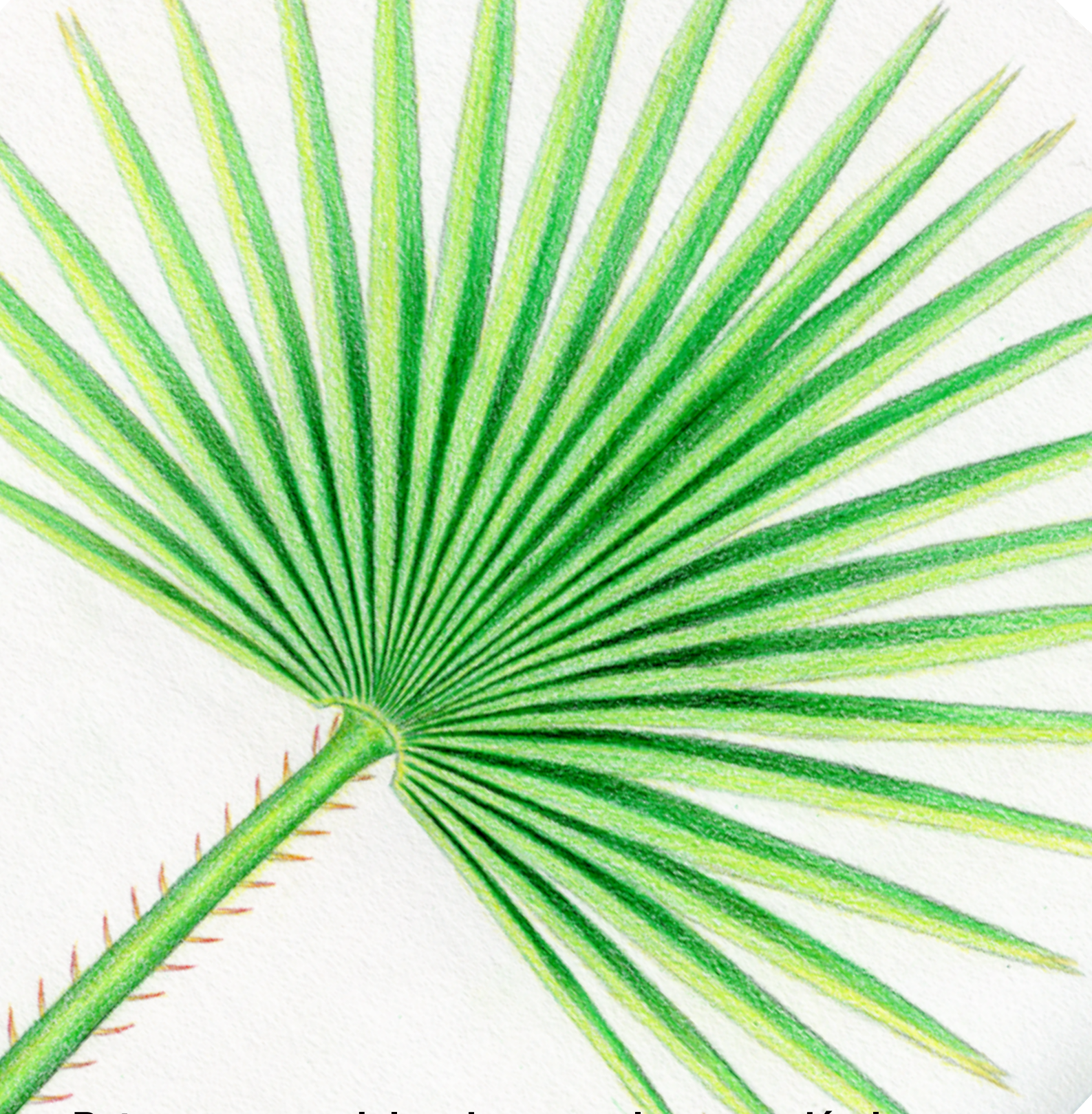

Patrones espaciales de una palmera endémica del Mediterráneo y sus efectos sobre la polinización y dispersión de semillas

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Tesis Doctoral, 2015

A Angeles y Miguel, Gemma y amigos

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Doctorado en Estudios Medioambientales

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Memoria presentada por el Licenciado en Biología Miguel Eduardo Jácome Flores para optar al título de Doctor por la Universidad Pablo de Olavide

Fdo. Miguel Eduardo Jácome Flores

Dr. Miguel Delibes y Dr. Jose Mª Fedriani

Estación Biológica de Doñana (CSIC)

CERTIFICAN

Que los trabajos de investigación desarrollados en la Memoria de Tesis Doctoral "Patrones espaciales de una palmera endémica del mediterráneo y sus efectos sobre la polinización y dispersión de semillas" son aptos para ser presentados por el Ldo. Miguel Eduardo Jácome Flores ante el Tribunal que en su día se designe, para aspirar al Grado de Doctor por la Universidad Pablo de Olavide.

Y para que así conste, y en cumplimiento de las disposiciones legales vigentes, extendemos el presente certificado a 13 de octubre de 2015.

Directores:

Fdo. Miguel Delibes

Fdo. Jose Mª Fedriani Laffitte

Tutora:

Fdo.Antonia Jiménez

“Después de andar tanto por España y de penetrar un poco -tal es mi intención en el alma del pueblo, todo podría ocurrírseme menos compadecer a los niños del coto de Oñana porque en su mundo encantado –de cuento de niños- no hay escuelas. Son felices. Se crían sanos. Saben lo que deben saber. Para ser como sus abuelos no necesitan más. (…) Para no variar nunca el orden preestablecido, lo mejor es que siga en libertad, aprendiendo el lenguaje de los pájaros y las alimañas. ¡Tierra singular, paradisíaca, primitiva, imposible sobre cualquier otro rincón de Europa!“.

Luis Bello, 1928, ed.1998: 267

ÍNDICE

RESUMEN

Los patrones espaciales de las plantas son el resultado de la interacción de tres factores: las condiciones ambientales donde se distribuyen, las presiones antropogénicas y las diversas interacciones ecológicas interespecíficas e intraespecíficas. Asimismo, estos patrones afectan a la capacidad de las plantas para usar los recursos necesarios para su supervivencia y las interacciones que mantienen con otras especies. La distribución espacial de las plantas puede tener efectos positivos, neutros y negativos sobre el éxito reproductivo, operando de manera dinámica a lo largo del ciclo reproductivo de los individuos. Por ejemplo, plantas aisladas pueden ser menos polinizadas que las plantas agregadas, pero los frutos de las últimas pueden experimentar una mayor depredación.

En esta tesis doctoral se evalúan los efectos de los patrones espaciales del palmito *Chamaerops humilis* sobre las distintas interacciones que esta planta mantiene a lo largo de su ciclo biológico. Para poder abordar con eficiencia este objetivo general, en el **capítulo 1** nos centramos en caracterizar y cuantificar los patrones espaciales del palmito mediante técnicas de análisis espacial de patrón de puntos. Encontramos que los palmitos siguen una distribución agregada, caracterizada por agregaciones de "gran tamaño" de plantas femeninas y masculinas las cuales, a su vez, están conformadas por pequeñas agregaciones de individuos, así como algunas plantas distribuidas al azar. Este patrón complejo parece ser resultado de los distintos comportamientos de los diversos dispersores de semillas de la especie. Aunque lógicamente otros factores bióticos (e. g. herbivoría de plántulas y depredación de semillas) y abióticos (e. g. condiciones de hábitat estresantes, incendios, presiones antropogénicas) también pueden haber contribuido a dicho patrón espacial.

En los capítulos 2, 3 y 4 evaluamos cómo los patrones espaciales afectan a las interacciones que esta especie mantiene con distintos animales mutualistas y antagonistas, específicamente con los polinizadores, dispersores de semillas y depredadores de semillas. En lo que respecta a la polinización, el palmito mantiene una relación mutualista muy especializada con el gorgojo *Derelomus chamaeropsis* denominada *nursery pollination,* en la cual la planta provee al gorgojo de cobijo para el desarrollo de sus larvas a cambio de los servicios de polinización. El éxito de polinización del palmito dependerá en buena medida del número de polinizadores que las plantas

puedan producir a costa de sus estructuras florales, lo que requiere que polinizadores y flores se encuentren en el tiempo y el espacio. Por tanto, en el **capítulo 2** nos centramos en evaluar cómo los patrones temporales y espaciales de floración influyen sobre la presencia y abundancia de las larvas del gorgojo polinizador *D. chamaeropsis*. Primeramente encontramos que los palmitos masculinos tienen una probabilidad de tener larvas de más del 70%, mientras que las inflorescencias femeninas cae al 30%. Asimismo, los palmitos masculinos con valores altos de sincronía floral y pocas inflorescencias tuvieron mayor abundancia de larvas que los palmitos femeninos. Espacialmente, a pesar de que los palmitos siguen una distribución agregada, encontramos que la presencia y abundancia de larvas del gorgojo no se vio afectada por la estructura espacial de la planta huésped, siguiendo una distribución aleatoria. En el **capítulo 3** analizamos las consecuencias del patrón espacial del palmito sobre el éxito temprano y tardío de la polinización. En este capítulo utilizamos los resultados del capítulo 1 para distinguir entre plantas agregadas y poco agregadas, en las que evaluamos las posibles limitaciones en la calidad del polen mediante experimentos de polinización manual. Asimismo de forma observacional medimos el éxito de polinización con respecto a la agregación de las plantas, para lo cual analizamos la estructura genética espacial de las poblaciones objetivo. No encontramos ningún efecto de la agregación espacial de las plantas sobre el éxito de polinización, quizás debido a que la distancias entre plantas (plantas muy agregadas y plantas poco agregadas) nunca fue mayor a la distancia a la cual los gorgojos pueden transportar polen. Sin embargo, parece ser que la cantidad de polinizadores que llegan al palmito es insuficiente, ya que el éxito de polinización temprano fue menor en las inflorescencias polinizadas naturalmente. Por otro lado, a pesar de que los palmitos tienen una distribución altamente agregada, el análisis genético evidenció que los individuos están poco emparentados, lo que explicaría el alto porcentaje de fructificación tardía sin importar el grado de agregación en las plantas. Finalmente en el **capítulo 4** evaluamos si los patrones de agregación espacial afectaban a las tasas de visita y consumo de frutos de distintos grupos funcionales de frugívoros (dispersores, despulpadores y depredadores de semillas). En este capítulo tampoco hallamos que la distribución espacial (plantas agregadas vs. aisladas) afectara a la frugivoría en general. Sin embargo, fuimos capaces de detectar que los palmitos experimentan limitación en la dispersión, pues un gran porcentaje de frutos no son dispersados debido a la alta depredación por ungulados, y otro porcentaje es dispersado ineficientemente por conejos y roedores a muy cortas distancias. Igualmente, observamos que el tejón es el frugívoro de mayor

relevancia para el éxito reproductivo del palmito, ya que realiza la dispersión de semillas a largas distancias, promoviendo la colonización de nuevos hábitats y la conectividad entre parches.

En resumen, los resultados de esta tesis indican que la agregación espacial del palmito da lugar a distintos mecanismos de denso-dependencia que afectan de forma diversa a sus interacciones con animales mutualistas y antagonistas. Esta investigación deja en claro que para entender el efecto neto de la denso-dependencia espacial hay que examinar de forma detallada su efecto en cada una de las fases del ciclo reproductivo de la planta. Cualquier alteración en los patrones espaciales y en la densidad de plantas por efecto de la fragmentación se traduciría en una alteración de la presencia o abundancia de las especies animales con las que interactúa. En el caso de la polinización, la reducción y el aislamiento de las poblaciones podría causar depresión endogámica, una reducción del fitness de las plantas y por último una disminución en la viabilidad general de la población. En cuanto a la dispersión, la disminución de las poblaciones de dispersores legítimos limita los servicios de dispersión a larga distancia, la colonización de nuevas áreas y la regeneración de las poblaciones.

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INTRODUCCIÓN GENERAL

La actual crisis de biodiversidad se manifiesta con la perdida de millones de poblaciones animales y vegetales al año (Hughes et al. 1997, Krauss et al. 2003, Green et al. 2005). En buena medida, dicha crisis ha sido originada por actividades humanas tales como la reducción de hábitats, la fragmentación y la sobreexplotación de los recursos (Tilman et al. 2001, Foley et al. 2005, Green et al. 2005). Además de la extinción de muchas poblaciones, esto ha ocasionado el declive de muchas otras de pequeño tamaño, donde los procesos de denso-dependencia afectan a su supervivencia y tamaño poblacional (Strong 1986, Hixon et al. 2002). Se ha constatado como en poblaciones con baja densidad de individuos, una pérdida de los ya limitados efectivos poblacionales deriva en un declive de la supervivencia y también del crecimiento de la población (denso-dependencia directa o positiva). Por el contrario, en poblaciones con altas densidades una disminución en el número de individuos normalmente produce una respuesta positiva tanto a nivel individual como poblacional (denso-dependencia inversa o negativa) debido a una disminución de la competencia intraespecífica (Courchamp et al. 2008).

Como otros muchos procesos ecológicos, los mecanismos denso-dependientes pueden suceder a lo largo de diferentes fases del ciclo reproductivo de los individuos y son dependientes de los patrones espaciales (Fedriani et al. 2015). Estos patrones espaciales de las plantas son resultado de su propia capacidad para producir y dispersar semillas, la competencia intraespecífica, la competencia interespecífica, las perturbaciones, la herbivoría y la heterogeneidad ambiental (Phillips and Macmahon 1981, Eccles et al. 1999, Tirado and Pugnaire 2003, Schurr et al. 2004, Fedriani et al. 2010). Por tanto, el análisis de los cambios en los patrones espaciales en el tiempo puede ayudar a valorar los efectos del éxito reproductivo (polinización, dispersión y establecimiento de plántulas) dependiente de la densidad.

La forma más frecuente de describir los patrones espaciales se basa en la premisa de la autocorrelación espacial, en la que se establece que los individuos de sitios cercanos van a ser mas similares entre si que aquéllos que se encuentren a una mayor distancia (e. g., Levin 1992, Wiegand et al. 2003, 2007, 2009, Mcintire and Fajardo 2009). Tomando en cuenta esta dependencia espacial, durante los últimos 20 años se han desarrollado un conjunto de técnicas para

analizar los patrones espaciales de las poblaciones vegetales (Elith and Leathwick 2009, Dormann et al. 2007, Dungan et al. 2002, Legendre et al. 2002, Turner 2005). El análisis espacial de patrón de puntos (SPPA por sus siglas en inglés) es una técnica estadística que utiliza la localización geográfica de todas las plantas dentro de un área delimitada para cuantificar las relaciones espaciales entre los individuos. El SPPA permite una descripción precisa de las propiedades de los patrones espaciales, ya que no sólo describe si los individuos se encuentran de forma aleatoria o agregada sino que puede proporcionar datos objetivos sobre el número de agregaciones, la escala espacial a la que éstas se encuentran, su tamaño, la existencia de superposición de diferentes patrones espaciales e inclusive las asociaciones espaciales de distintos elementos del fitness de cada individuo (tamaño, sexo, número de frutos, etc.) (Wiegand et al. 2007, Fedriani et al. 2010, 2015).

Los patrones espaciales afectan no sólo a cómo la especie utiliza los recursos, sino también la forma en la que ésta es utilizada como recurso por otras especies con las que interactúa, como polinizadores, depredadores y dispersores de semillas (Ghazoul 2005, Aizen and Vázquez 2006, Muller-Landau et al. 2008, Fedriani y Wiegand 2014, Wiegand et al. 2009). Por ejemplo, plantas aisladas pueden ser menos visitadas por polinizadores, disminuyendo el cuajado de frutos (denso-dependencia positiva; Nielsen and Ims 2000, Aizen and Vázquez 2006), pero experimentar una menor depredación de frutos (denso-dependencia negativa; (Janzen 1970, Connell 1971, Kolb et al. 2007, Mezquida and Olano 2013). Es por ello que en esta tesis nos centraremos en cómo los procesos de denso-dependencia y patrones espaciales afectan a las interacciones planta-animal en diferentes etapas del ciclo reproductivo de la planta.

Los patones espaciales y las interacciones planta-animal

La polinización

La carencia de movilidad de las plantas representa un obstáculo para la dispersión de sus genes, por lo que la mayoría tuvo que desarrollar estrategias para lidiar con esta limitante. Una de las estrategias más exitosas fue establecer relaciones mutualistas con animales, principalmente insectos, quienes a cambio de polen y néctar transportan los granos de polen entre estigmas,

logrando la fertilización y el desarrollo de los frutos (Howe and Smallwood 1982, Herrera 2002, Pellmyr 2002, Lopez and Vaughan 2004). En muchos sistemas de polinización el nivel de agregación de las plantas determina la tasa de encuentro exitoso entre la planta y su insecto polinizador a través del paisaje (Aizen and Vázquez 2006, Stehlik et al. 2006, García-Camacho et al. 2009). Varios estudios han demostrado que los insectos polinizadores se mueven distancias relativamente cortas, por lo que es menos probable que encuentren, visiten o gasten tiempo en parches con baja densidad de plantas que en parches con alta densidad, con individuos bien conectados entre si (Klinkhamer et al. 1989, Robertson and MacNair 1995, Gascoigne et al. 2009, Fedriani et al. 2015). Asimismo, los polinizadores por lo general se sienten mas atraídos a zonas con altas densidad donde exista una gran cantidad de recursos, no sólo porque ofrecen más recursos florales en conjunto, sino porque la distancia entre plantas es reducida, disminuyendo los costes energéticos de movilidad e incrementando la tasa de visitas por flor y el cuajado del frutos (Kunin 1993, 1997, Cartar and Real 1997, Bosch and Waser 2001). Es decir, una reducción en la densidad de plantas disminuiría la capacidad de atracción de polinizadores, reduciendo la cantidad de eventos de polinización y el éxito de polinización (Nielsen and Ims 2000, Aizen and Vázquez 2006). De hecho, en el meta-análisis realizado por Aguilar et al. (2006) sugieren que la reducción de las poblaciones y la fragmentación son los procesos que mas influyen sobre el fracaso de polinización de las especies. Sin embargo, en poblaciones con altas densidades no siempre operan efectos de denso-dependencia positiva, ya que puede ser que las agregaciones estén formadas por individuos altamente relacionados entre sí, disminuyendo la calidad del polen y el cuajado final de frutos (Ishihama et al. 2006, Aizen and Harder 2007). Asimismo, la polinización en sitios con altas densidades se puede ver afectada por competencia interespecífica por recursos o competencia intraespecífica por polinizadores (Gunton and Kunin 2009, Spigler and Chang 2009).

La dispersión de semillas

En cuanto a la dispersión de semillas, las plantas también han establecido distintos mutualismos con varios grupos animales (e. g. aves, murciélagos, lagartijas, carnívoros). Una de ellos es la endozoocoria o el transporte de semillas dentro del sistema digestivo hacia otras áreas, generalmente lejos de las plantas madre (Fleming and Estrada 1993, Jordano et al. 2011). Esta estrategia, según la hipótesis de Janzen-Connell (Janzen 1970, Connell 1971), aumenta las

probabilidades de supervivencia de las semillas y las posibles plántulas, ya que ambas pueden escapar de parásitos y depredadores asociados a la planta madre (Rodríguez et al. 2014), evitan la competencia intraespecífica y además aumentan la probabilidad de establecerse en un hábitat propicio para su supervivencia (Nathan and Muller-Landau 2000, Wang and Smith 2002, Terborgh et al. 2008).

Por supuesto, no todos los animales frugívoros son dispersores de semillas y existen cuando menos tres grupos funcionales de frugívoros clasificados según su capacidad para dispersar semillas: dispersores legítimos (transportan las semillas sin dañar lejos de la planta madre), dispersores ineficientes (remueven eficientemente la pulpa, pero dejan las semillas cerca de la planta madre) y depredadores de semillas (Fedriani and Delibes 2013). La intensidad de interacción de estos grupos funcionales con las plantas (i.e. número de frutos consumidos) se ve ampliamente afectada por los patrones espaciales (Levey et al. 1984, Saracco et al. 2004). Los frugívoros se ven atraídos hacia plantas con un patrón agregado donde los recursos son más abundantes, lo que podría tanto incrementar la tasa de consumo de frutos (García et al. 2001, Moegenburg and Levey 2003, Takahashi and Kamitani 2004, Carlo and Morales 2008) como disminuirla, por algún efecto de saciedad de los frugívoros o competencia intraespecífica (Moore and Willson 1982, Manasse and Howe 1983, Denslow 1987).

La dispersión de semillas también puede verse afectada por la reducción del hábitat y la defaunación selectiva. La reducción de las poblaciones de plantas significa que serán menos atractivas para los dispersores, y la reducción de los dispersores por defaunación actuará de forma sinérgica reduciendo aún más las probabilidades de dispersión, resultando en la pérdida de funciones mutualistas y poniendo en peligro a las especies conectadas (Wang et al. 2007, Vanthomme et al. 2010, Galetti and Dirzo 2013).

Finalmente, y en secuencia con el ciclo reproductivo de las plantas, los patrones espaciales también pueden afectar a la emergencia, supervivencia, crecimiento y establecimiento de plántulas. Se ha visto que los parches con densidades altas de plantas o muy agregados atraen a una mayor cantidad de depredadores que buscan brotes tiernos o plántulas emergentes (Fedriani and Delibes 2011). Esto se relaciona con la hipótesis de Janzen-Connell, según el cual las semillas

y plantas jóvenes sufren una mayor mortalidad en la vecindad de la planta madre (Janzen 1970, Connell 1971). Varios estudios han demostrado que la supervivencia de plántulas disminuye al aumentar la densidad, tanto de plántulas como de adultos conspecíficos (Webb and Peart 1999, Harms et al. 2000, Metz et al. 2010).

En resumen, los cambios en los patrones espaciales por eventos de fragmentación o reducción de las poblaciones vegetales pueden afectar de manera negativa a la supervivencia de las plantas en cualquiera de las etapas de su ciclo de vida. Infortunadamente, la ocurrencia de estos procesos y sus efectos cada vez es mayor. Un claro ejemplo es el matorral mediterráneo, que durante la segunda mitad del siglo XX ha experimentado fuertes cambios de usos de suelo, fragmentación y reducción de hábitat, y defaunación (Kosmas et al. 1997, Stoate et al. 2001, García-Ruiz 2010), con severas consecuencias sobre la supervivencia de las plantas que lo componen (Forget and Jansen 2007). Una de las especies que más afectada se ha visto y menos atención ha recibido es el palmito *Chamaerops humilis,* especie de palmera que, y junto con, la palmera *Phoenix theophrasti* son las únicas palmeras nativas de Europa. El palmito es una especie clave dentro del ecosistema mediterráneo, de la cual dependen varios procesos ecológicos. Sin embargo, existe una rápida degradación y reducción del ambiente donde se distribuye, sobre todo en las costas de Italia, Francia y España. Un claro ejemplo de esto es la distribución altamente fragmentada del palmito dentro del Parque Nacional de Doñana, donde las diversas presiones antropogénicas (cultivos, urbanizaciones) han derivado en la reducción de la población de la especie y de las especies con las que interacciona. Debido a todo lo anterior, es esencial el estudio de la relación entre los patrones espaciales del palmito y sus consecuencias sobre las interacciones planta-animal para el desarrollo eficaz de estrategias para su conservación.

OBJETIVOS DE LA TESIS DOCTORAL

1. En el **Capítulo 1** se cuantifican y analizan los patrones espaciales de los palmitos en dos zonas en las que están presentes en bajas densidades y se encuentran en su frente de colonización. A su vez, los resultados obtenidos en este capítulo servirán para analizar en los siguientes capítulos el efecto de la estructura espacial en las interacciones planta-animal..

2. En el **Capítulo 2** se analiza cómo la sincronía floral, el sexo de las plantas y los patrones espaciales del palmito influyen sobre la presencia y abundancia de su polinizador, el gorgojo *Derelomus chamaeropsis*.

3. En el **Capítulo 3** evaluamos observacional y experimentalmente la influencia de los patrones espaciales y la densidad de plantas sobre el éxito de polinización temprano (número de drupas cuajadas) y tardío (número de frutos totalmente desarrollados).

4. En el **Capítulo 4** analizamos cómo la distribución espacial del palmito afectan a la tasa de visita y consumo de frutos de los distintos grupos funcionales de frugívoros y sus consecuencias sobre la dispersión de semillas.

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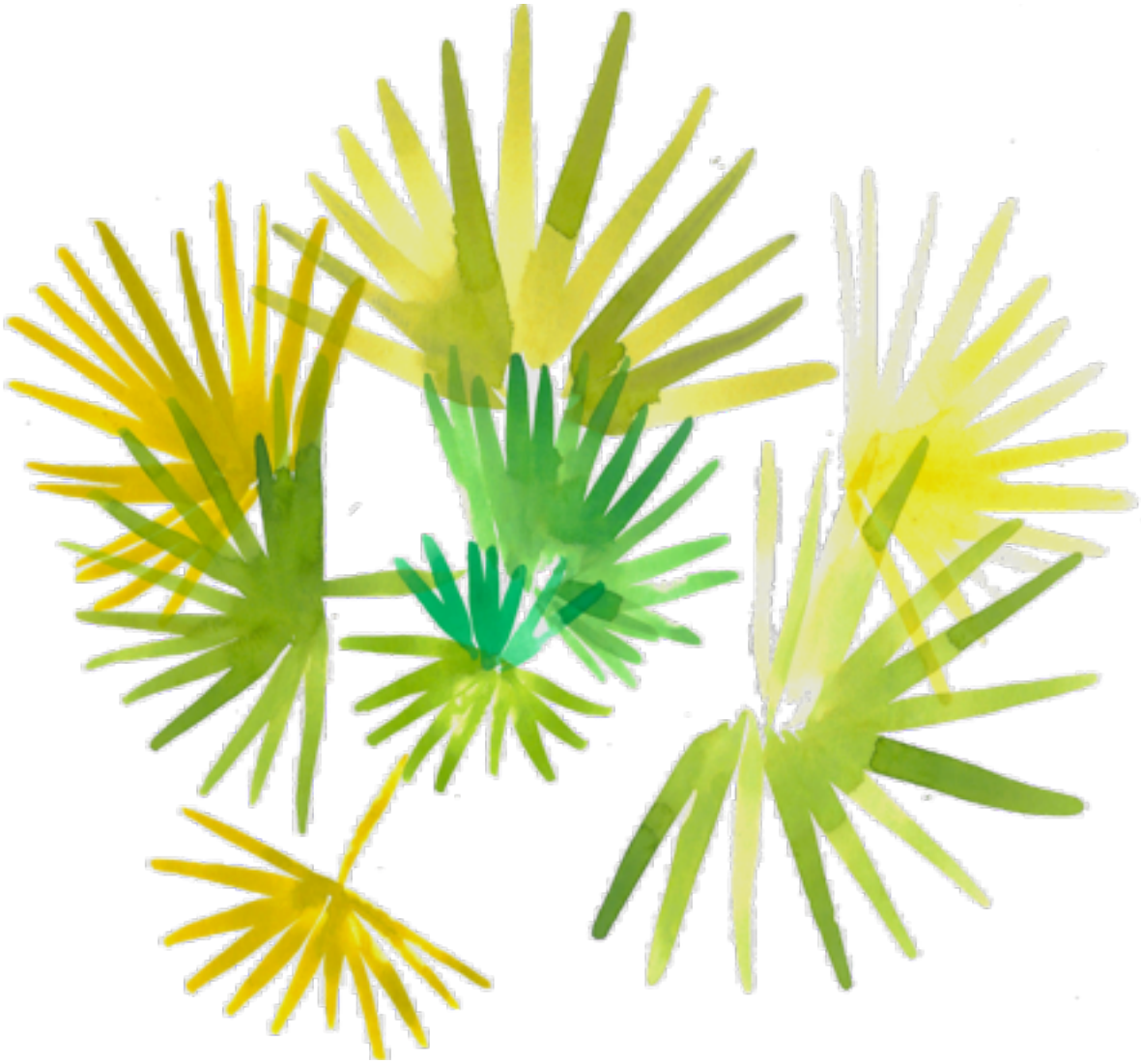
CAPÍTULO 1:

SPATIAL PATTERN OF AN ENDEMIC MEDITERRANEAN PALM AT ITS COLONIZATION FRONT



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RESUMEN

Los patrones espaciales de las plantas, con dispersión endozoócora, en su frente de colonización se ven influenciados tanto por los distintos comportamientos de sus dispersores como por las condiciones ambientales estresantes. La cuantificación de los patrones espaciales nos ayuda a inferir los procesos subyacentes que los generan, y consecuencias sobre la supervivencia de las plantas. Analizamos la estructura espacial de dos poblaciones del palmito *Chamaerops humilis* en su frente de colonización, utilizando distintas técnicas del análisis espacial de patron de puntos. En ambas poblaciones georreferenciamos todos los individuos adultos dentro de parcelas de 21 ha. Utilizamos el proceso de Thomas para describir los proceso de agrupación, etiquetado aleatorio para analizar la asociación espacial sexos y la función correlación de marca para analizar la estructura espacial de las tallas de las plantas. En ambas parcelas encontramos que las plantas estaban agregadas a dos escalas, donde, las grandes agregaciones (38-44 m) estuvieron compuestas por agregaciones pequeñas (2.8-4 m). Asimismo, encontramos que entre el 11-27 % de los palmitos se encontraban distribuidas aleatoriamente dentro de las parcelas. Estos hallazgos pueden ser explicados por las distintas estrategias de dispersion y abundancia relativa de los animales que dispersan el palmito. También, encontramos que los sexos tienen una distribución aleatoria. Esto fue consistente con la falta de segregación de las tallas de los palmitos de ambos sexos, lo cual sugiere que no existen diferencia en las tasas de supervivencia y crecimiento entre plantas de distinto sexo. Las tallas mostraron agregación espacial dentro de las agrupaciones con un decrecimiento con la distancia. En general, nuestros resultados muestran que los patrones de agrupación podría tener un efecto positivo la colonización del palmito, dependiendo de los dispersores de semillas y las condiciones ambientales.

ABSTRACT

Spatial patterns of endozoochorous plants at its colonization front should be influenced by distinctive behaviors of their disperser and stressful environment conditions of the new habitat. A precise quantification of the spatial patterns could help us to infer some of the underlying processes generating them, as well as to predict their consequences for the palm reproductive performance. We used different techniques of point pattern analysis (SPPA) to quantify the spatial structure of two *Chamaerops humilis* populations at their colonization front, discussing the potential processes generating such patterns and their likely consequences on palm reproductive success. In each population we mapped all adult *C. humilis* individuals at a 21-22 ha plot. We used Thomas process to describe the clustering process, random labeling to analyze the sexes spatial association and mark correlation for plant size spatial structure. Plants in both plots showed two critical scales of clustering, whith large clusters (38-44 m) composed by small ones (2.8-4 m). Additional to the clustered individuals, 11% and 27% of all dwarf palms belonged to a random pattern independently superimposed to the clustered component pattern. This finding could be explained by the effect of different seed disperser and predators' behavior and their relative abundance. Plant sexes had not spatial segregation, where females and males composed cluster equally. This was consistent with the lack of segregation of female and male sizes, suggesting similar survivorship and growth rates. Sizes showed a spatial segregation inside the clusters, with a decreasing correlation with distance where the clustering pattern dilutes. Overall, our results show that plants clustering patterns have an effect over its colonization front, which is strongly reliant on seed dispersers and environment stressful conditions.

INTRODUCTION

The spatial pattern of plants is an essential ecological attribute with important implications on their growth, reproductive success, and survival (Prentice and Werger 1985, Fowler 1988, Condit et al. 2000, Novoplansky and Goldberg 2001). Plant reproduction is particularly sensitive to potential contingencies of spatial distribution (Stehlik et al. 2006) and, because of this, plants within a patch tend to have similar reproductive successes (Klinkhamer et al. 1987, Wesselingh et al. 1997, Méndez and Karlsson 2004). Also, the level of population aggregation is critical for plant interactions with both mutualistic and antagonistic animal associates, such as pollinators (Aizen and Vázquez 2006, Nielsen et al. 2006), seed predators (Bonal et al. 2006, Muller-Landau et al. 2008, Wiegand et al. 2009, Fedriani et al. 2010), seed dispersers (Malo and Suárez 1995, Silvius and Fragoso 2002, Fedriani and Delibes 2011) and seedling predators (Augspurger 1983, Velho et al. 2012, Gripenberg et al. 2014). High density of conspecific neighbors may, for example, facilitate encounter by antagonists (Silvius and Fragoso 2002, Fedriani et al. 2012) or result in predator satiation and thus attenuate the *per capita* risk of predation (Bonal et al. 2006, Jones and Comita 2010). Therefore, characterizing in detail the spatial pattern of plant populations is needed to comprehensively understand the mechanisms underlying their variation in fitness components (e.g., (Levin 1992, Wiegand et al. 2003, 2007c, Wiegand and A. Moloney 2004, Mcintire and Fajardo 2009, Fedriani et al. 2015).

The spatial distribution of plants is often codetermined by seed dispersal and environmental and historical factors impinging on plant arrival, establishment and survival (Gartlan et al. 1986, Castro et al. 2005). For instances, in the case of endozoochores, adult plant spatial pattern could show signatures of the spatial patterning of seed dispersal (e.g. from highly scattered to highly aggregated), that is strongly influenced by the disperser movement and spatial fecal marking behavior (Schupp et al. 2002, 2010, Wiegand et al. 2007a, Fedriani and Wiegand 2014). Furthermore, the establishment of new individuals could be affected by local environmental conditions such as moisture (Villers-Ruiz et al. 2003), soil type (Shaukat et al. 2012), rainfall (He et al. 2014) or temperature (Villers-Ruiz et al. 2003). Also, human-related historical factors (e.g. plant removal, fire, fragmentation) provide another plausible explanation for certain spatial patterns (e.g. Camarero et al. 2005).

The spatial distribution pattern of well-established plant populations has been intensively investigated in both tropical and temperate habitats (Wiegand et al. 2009, Raventós et al. 2010, Shaukat et al. 2012, Fedriani and Wiegand 2014). Surprisingly, however, very little is known concerning plant spatial patterns, and their causes and consequences,at the population colonization front, where plants usually occur at low-density due to the prevalence of stressful ecological conditions that reduce plant growth, survival and reproduction, compared with large populations (Chhin and Wang 2002, Camarero et al. 2005). Populations at its colonization front may experience poorer plant and pollinator faunas (Silva-Montellano and Eguiarte 2003, Stone and Jenkins 2008), pollen limitation due to difficulty in finding a mate (Bessa-Gomes et al. 2003), limited seed dispersal (Travis and Dytham 1999, Holt 2003) and inbreeding depression because of an insufficient number of founders (Ovaskainen and Hanski 2001). In this context, it is particularly interesting to characterize quantitatively the spatial patterns of low-density plant populations at their colonization fronts, as this can shed light about the ecological, environmental and historical factors leading to it.

Spatial point pattern analysis (SPPA; (Stoyan and Stoyan 1994, Diggle et al. 2003, Illian et al. 2008, Wiegand and Moloney 2014) is an statistical technique that allows for a detailed characterization of the smaller-scale spatial distribution pattern of “ecological objects” such as plants. More generally, spatial point pattern data consist of the georeferenced locations (point) of every plant within a study plot, which can be supplemented by additional information characterizing the points (i.e., marks such as size or surviving vs. dead). Of special interest in plant populations is to characterize the way they are spatially clustered. Thomas processes are a class of relatively simple point process models that proved to be suitable for describing clustering in natural plant populations (e.g. Fedriani and Wiegand, 2014; Wiegand et al., 2009). In the simplest case of a Thomas process, the point pattern is assumed to consist of a number of independently distributed clusters where the plants are scattered with a two-dimensional normal distribution around the cluster centers. The fitted parameters then provide a succinct description of the characteristics of the observed pattern, given a good fit (Wiegand et al. 2007c, 2009), and allow for insight on the processes that structure the populations. The Thomas processes can also be extended to consider two critical scales of clustering that may be caused, for example, by two

subsequent mechanisms of seed dispersal (Wiegand et al. 2009). This allows for a very realistic representation of complex spatial patterns.

Finally, marked SPPA techniques can be used to analyze the spatial correlation structure of plants traits (e.g., a mark “male” vs. “female”, or size; Illian et al. 2008, Jacquemyn et al. 2010, Fedriani et al. 2015). In dioecious species, sexual spatial segregation (SSS) adds complexity to the spatial patterns and has been repeatedly observed in various species (e.g. Bierzychudek and Eckhart, 1988; Eppley, 2005). It has been described that SSS can be due to females preferring less stressful areas of the environment compared to males (Eppley 2005, Reuss-Schmidt et al. 2015), to differential germination (Eppley 2001) or to differential mortality between sexes (e.g. Gibson and Menges, 1994; Meagher, 1982). Finally the size of the plants is frequently associated in the space (Moeur 1997). Nakagawa et al. (2015) found that aggregations are mostly composed by similar larger plants that compete and remove medium-sized neighbors. This is influenced by perturbation, neighboring plant populations in stressful habitats having a bigger variety of plant sizes and ages compared to less perturbed areas (Weiner et al. 2009).

In this study, we used SPPA to quantify the spatial distribution patterns at the colonization front of two low-density populations of dwarf palm *Chamaerops humilis* differing in management history. This palm species, relatively abundant in Mediterranean scrub thickets and open pine forests, is endemic to the Western Mediterranean basin, with no obvious preference of type soil or substratum (Herrera 1989). Recently, anthropogenic pressures and the introduction of noxious pest have drastically reduced dwarf palm populations in part of its distribution range (Drescher and Dufaÿ 2001, Rodríguez et al. 2014). We characterized in detail the spatial distribution patterns of two palm populations at their colonization front in a range of spatial scales, discussing the potential processes generating such patterns and their likely consequences on palm reproductive success. Comparison of detailed characteristics of spatial patterns of the same species at two different sites will allow us to determine common drivers of the patterns and evaluate the impact of site differences. In particular, we tested the following three hypothesis: 1) Because both long- and short-distance seed dispersers interact with *C. humilis* (Fedriani and Delibes 2011), we expect that both populations will show a spatially aggregated pattern with several critical scales, 2) both female and male dwarf palms will occupy equally plant aggregations, due to high tolerance of

both sexes to stressful conditions and 3), because plants in perturbed areas could have a big variety of sizes (Weiner et al. 2009), we expect that dwarf palms at both populations will have no spatial segregation by size.

MATERIAL AND METHODS

Study species and area

Chamaerops humilis is a small (usually ~1.5 m high) dioecious palm (Herrera 1989), considered a thermo-mediterranean bioindicator. In Europe, it is usually not present beyond 1000 meters above sea level, being most common in coastal areas. In Spain and Morocco we still can find well-establish populations, where the species reach high densities and even form "monodominant" patches of several hectares (Fedriani & Delibes, 2011, Authors personal observations). Due to its vigorous sprouting, *C. humilis* is very tolerant to disturbance (fire, herbivory, etc; Herrera, 1989) and thus it is often used in restoration programs in arid areas. It blooms during March-May showing a mixed insect and wind pollination system (Herrera 1989, Anstett 1999). Specifically, its main pollinator seems to be the host-specific palm flower weevil *Derelomus chamaeropsis* (Anstett 1999). The fruits are "polydrupes" comprising one to three drupes that ripe in autumn (September–November). Fruits are attached to infrutescences (or ramets) of up to 30 cm long (37–91 fruits per ramet; unpublished data).

In Europe populations the dispersal is almost exclusively by mammal species such as badgers (*Meles meles*), foxes (*Vulpes vulpes*) and rabbits (*Oryctolagus cuniculus*) (Fedriani and Delibes 2011). Finally, seedlings emerge during the spring and the early summer, experiencing extensive mortality due to both summer droughts and herbivory (Fedriani and Delibes 2009a, 2009b). Although the dwarf palm has been described by Herrera (1989) as a plant with no obvious preferences regarding type of soil or substratum, we found that low areas and marshes susceptible to be flooded lack dwarf palms (personal observation). In our study system, the Doñana Park, *C. humilis* is generally associated with sandy soils, presenting highly fragmented distributions due to both historic (crops, villages) and environmental (marshes, dune system) barriers.

The study was carried out in the Doñana National Park (510 km^2; 37º9' N, 6º26' W), located on the right bank of the Guadalquivir estuary in southwestern Spain. Average annual temperatures ranges between 15.37 and 18.67 ° C (mean = 16.91 ± 1.06 º C; n =35; period 1978-2013). Similarly, annual rainfall varied during this period between 170 and 1028 mm (mean=542.6 mm ± 12.02 mm; data from Monitoring Team of Natural Process of Doñana Biological Station; http://www-rbd.ebd.csic.es/Seguimiento/mediofisico.htm). Most rain was concentrated from October to March. Between November-December of 2011 we selected and delimitated two observational plots within Doñana area, called Matasgordas and Martinazo (Fig. 1), where we identified and georeferenced (with a submetric GPS) all adult reproductive *C. humilis* individuals (n= 399).

The vegetation and physiographic characteristics differ between plots. The Matasgordas plot is occupied by a dehesa, which includes some areas prone to flooding and is limited in the south by a marshland (Fig. 1). The dehesa habitat (~ 300 ha) was generated in 1970 when all shrubs and most trees were mechanically removed. This management resulted in a continuum of grasslands with open tree stratum of *Q. suber*, *O. europaea* var. *sylvestris* and *Fraximus angustifolia* with no, or only sparse, understory of Mediterranean scrubs (Fedriani et al. 2010). The area was used for intensive cow grazing until 1996, when the land became public and the cows were removed, under the protection of the Spanish National Park Service. Since then, several mammal-dispersed plants, including *C. humilis*, are recolonizing the area (Fedriani and Wiegand 2014). In this site we delimited a plot of 22.1 ha which included 308 adult individuals (Fig. 1B).

The Martinazo site is covered by a dense Mediterranean scrubland dominated by *Halimium halimifolium*, *Rosmarinus officinalis*, *Ulex* spp. and *Stauracanthus* spp. Historically, this area has been used for livestock ranching, which increased the herbivory pressure over the native shrub. Additionally woody species (*Quercus suber*, *Olea europaea* var. *sylvestris*) were cut down, and controlled rotating burnings were applied every 25-30 years (Granados et al. 1986, 1988). Since then, the area has been recolonized by vegetation composed mainly by a pyrophitic scrub (*Ulex* spp., *Stauracanthus* spp. and *C. humilis*) (Granados et al. 1988). Within the Martinazo site we delimitated a plot of 20.93 ha which included 91 *C. humilis* adult individuals (plants that had any flowering evidence) (Fig. 1C). The extension of this plot was determined by the marshland in the northeast part (Fig. 1C).

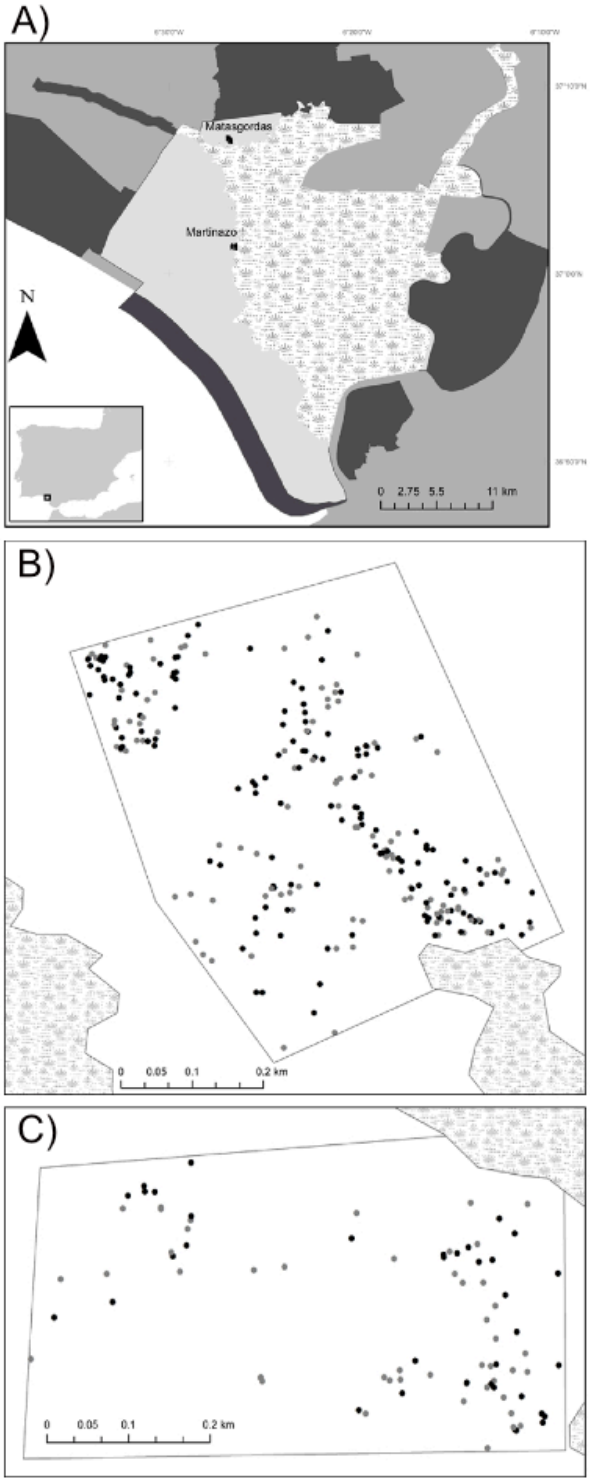

Figure 1. The two plots limited by the marshland (grass pattern) within the Doñana National Park area (lightest grey) (A). The plots (B) Matasgordas and (C) Martinazo with the georeferenced plants: the points in black represent female plants, and the gray points the males.

In both plots we georreferenced all adult *C. humilis* individuals (Fig. 1). For each plant we register the sex and the size. We identified the sex based in the inflorescences morphological differences: the females inflorescences are solid, brownish and had borne fruits or just the calyx, instead the males have smaller and very brittle inflorescences. The size (projected area of the plant canopy) was approached as the area of a ellipse (range 0.06-15.8 m^2), so we measured the minor and major radius. We conducted three types of analyses; first we used Thomas cluster point processes to characterize the observed plant clustering, second we use the random labeling null model to test if female and male individuals showed a non-random spatial pattern within all individuals, and finally we used mark correlation functions to investigate if the sizes of all plants, or those of males and females, showed spatial correlations.

Analysis of clustering

We used four classical point pattern summary statistics: the pair correlation function $g(r)$, the L-function $L(r)$, the spherical contact distribution $H_s(r)$, and the nearest neighbor distribution function $D(r)$ (Illian et al. 2008, Wiegand and Moloney 2014) to quantify the spatial pattern of the two study populations. For homogeneous patterns, the univariate pair-correlation function $g(r)$ can be defined as the density of points within a ring of radius r and width dw around the typical point of the pattern, divided by the intensity of the pattern. Thus, $g(r) > 1$ indicates clustering because the pattern shows a higher neighborhood density than expected by a random pattern (=). The L-function is the transformation $L(r) = (K(r)/\pi)^{0.5} - r$ of the K-function which is the cumulative version of pair correlation function, i.e., $K(r) = \int g(r)\, 2\pi r\, dr$. While the pair correlation function is especially sensitive to clustering at small scales, the L-function is more sensitive to clustering at larger scales. For a random pattern we find $L(r) = 0$ and for a clustered pattern $L(r) > 0$. The spherical contact distribution $H_s(r)$ yields the probability that a random "test" point has its first neighbor at distance r and characterizes the "holes" in the pattern. Conversely, the nearest neighbor distribution function $D(r)$, that characterizes the clustering of the pattern, returns the probability that the typical point of the pattern has its first neighbor at distance r.

Because the *C. humilis* plants were clearly clustered (Fig. 1), we used a suite of point processes to characterize in detail the properties of the clustering patterns. In a first step we fitted a Thomas process (Wiegand et al. 2007c, 2009) to the *L*- and pair correlation functions of the data that incorporated one critical scale of clustering. If this point process did not fitted well the summary statistics $g(r)$ and $L(r)$, we used in a second step a Thomas process that incorporates two critical scale of clustering. If this point process did not fit well the spherical contact distribution and the nearest neighbor distribution function (e.g., because the data contain more isolated point than predicted by this cluster process; Wiegand et al., 2007c), we used a point process that is an independent superposition of a Thomas process with two critical scale of clustering and a random pattern.

The Thomas process with one critical scale of clustering consists of randomly and independently distributed "clusters" where is the intensity of the cluster centers. The points of the pattern are then randomly assigned to the clusters, and their distribution relative to the cluster center follows a two-dimensional normal distribution with variance ^{2}. The cluster size r_C can be defined as r_C 2 and includes approximately 87 % of the points of a given cluster, and the approximate area covered by one cluster is $A_C = \pi r_C^2 = 4\pi^2$ (Wiegand et al. 2009) The pair-correlation function of this Thomas process yields (Wiegand et al. 2009):

$$g(r,\sigma,\rho) = 1 + \frac{1}{\rho}\frac{\exp(-r^2/4\sigma^2)}{4\pi\sigma^2} \quad (1)$$

The Thomas process with two critical scales follows the same construction principle as the one with one critical scale of clustering. The only difference is that the cluster centers do not follow a random pattern but are assumed to follow a Thomas process with one critical scale of clustering. This double-cluster process has four unknown parameters: the intensities $_L$ and $_S$ of the centers of the large and small clusters, respectively, and the parameters $_L$ and $_S$ that define the size of the large and small clusters, respectively. Its pair-correlation function yields (Wiegand et al. 2009):

$$g(r,\sigma,\rho) = 1 + \frac{1}{\rho_S}\frac{\exp(-r^2/4\sigma_S^2)}{4\pi\sigma_S^2} + \frac{1}{\rho_L}\frac{\exp(-r^2/4(\sigma_S^2+\sigma_L^2)}{4\pi(\sigma_S^2+\sigma_L^2)} \quad (2)$$

If the cluster centers approximate a random pattern (e.g., L is large), the third term of equation (2) disappears and the pair correlation function collapses to that of a Thomas process with one critical scale of clustering (eq. 1). Because equations 1 and 2 provide analytical solutions of the pair correlation functions (and thereby the *L*-functions), these point processes can be fitted using the standard minimum-contrast method based on $g(r)$ and $L(r)$ estimated from the data (see Wiegand et al., 2009 and section 4.1.4.3 in Wiegand & Moloney, 2014 for details).

The third point process we use here is an independent superposition of a random pattern with a Thomas process with two critical scales of clustering (Wiegand et al. 2007b, 2009). Denoting p_C as the proportion of the points belonging to the double-cluster process, the pair-correlation function of the superposition process yields:

$$g(r,\sigma,\rho) = 1 + \frac{p_C^2}{\rho_S}\frac{\exp(-r^2/4\sigma_S^2)}{4\pi\sigma_S^2} + \frac{p_C^2}{\rho_L}\frac{\exp(-r^2/4(\sigma_S^2+\sigma_L^2)}{4\pi(\sigma_S^2+\sigma_L^2)} \quad (3)$$

Thus, the functional form of the pair correlation function is identical to that of the cluster process (2), but the number of clusters is virtually elevated by factor $1/p^2_C$. Thus, fitting the $g(r)$ and $L(r)$ cannot determine the parameter p_C. However, superposition with a random pattern produces "isolated" points, which will affect both, the shape of $H_s(r)$ and $D(r)$. We therefore can use the spherical contact distribution $H_s(r)$ and the nearest neighbor distribution function $D(r)$ to determine the proportion p_C of random points.

Because the study sites showed irregular shapes, we used the Ohser edge correction described in detail in Wiegand and Moloney (2014, equations 3.29 and 3.30) based on the isotropized set covariance for irregularly shaped study areas. To obtain a good resolution of the small-scale clustering we used a bin of 0.5m and a ring width of 2.5m for estimation of the pair correlation function. Note that the cluster processes are stochastic processes and that different realizations of the same cluster process will yield somewhat different patterns (as shown by the

simulation envelopes of the simulated cluster processes). This means also that each realization would produce slightly different best-fit parameters when fitted with the generated cluster process. This also means that the fitted parameters of the observed data are only approximate estimates of the true underlying parameters.

Random labeling

To test for absence of spatial structure in the distribution of the two sexes we contrasted the observed data with the random labeling null model that shuffles the labels "male" and "female" randomly over the dwarf palms (Wiegand and Moloney 2014). We used several test statistics based on pair correlation functions to test for departures from random labeling (Jacquemyn et al. 2009):

- $p_{11}(r)$*: tests if females show at distance r a pattern within all dwarf palms.*
- $p_{12}(r)$*: tests if males at distance r are spatially associated with females*
- *dif(r): tests if females are surrounded at distance r by a higher dwarf palm density than males.*

The test statistic $p_{11}(r)$ is the univariate mark connection function which yields the probability that of two randomly selected dwarf palms which are distance r apart both are females. The expectation of $p_{11}(r)$ under random labeling yields $p_{11}(r) = p_1^2$. For $p_{11}(r) > p_1^2$ the females are clustered at distance r within all palms where p_1 is the proportion of females among all dwarf palms.

The test statistic $p_{12}(r)$ is a bivariate mark connection function, which yields the probability that of two randomly selected dwarf palms, which are distance r the first is female and the second male. The expectation of $p_{12}(r)$ under random labeling yields $p_{12}(r) = p_1\ p_2$. For $p_{11}(r) < p_1\ p_2$ females and males are segregated at distance r within all palms (p_2 is the proportion of males among all dwarf palms).

Finally, the test statistic *dif*(r) compares the overall neighborhood density of dwarf palms at distance r around females with that around males and yields $dif(r) = g_{1,1+2}(r) - g_{2,1+2}(r)$. If $dif(r) > 0$ females are located in areas of higher palm density than males.

Mark correlation function

To find out if the sizes of both female and male individuals of *C. humilis* located distance r away were positively correlated, we used the framework of mark correlation function (Illian et al. 2008, Wiegand and Moloney 2014). Our data comprise for each individual the coordinates, the sex (male or female) and the mark "size". The bivariate mark correlation functions then consider all pairs of male and female palms (with index i, j and their marks m_i and m_j, respectively), selects those pairs with inter-point distance r, and estimate the mean of a test function $t(m_i, m_j)$ over these pairs which is then divided by the expectation of the function over all pairs i-j.

The r-mark correlation function $k_{.m}(r)$ uses the test function

$$t(m_i, m_j) = m_j \qquad (4),$$

and estimates therefore the mean size $\mu_f(r)$ of females j of each female-male pair i-j with inter-point distance r divided by the mean size μ_f of the females, i.e., $k_{.m}(r) = \mu_f(r)/\mu_f$ (Illian et al. 2008). Thus, $k_{.m}(r) > 1$ indicates that females that have males at distance r are on average larger than expected. Conversely, $k_{m.}(r) < 1$ indicates that females, which have males at distance r, are on average smaller than expected.

We are also interested in the correlation between the sizes of male and female palms that are distance r apart. The appropriate test function for this purpose was proposed by Schlather et al., (2004):

$$t(r, m_i, m_j) = [m_i - \mu_m(r)][(m_j - \mu_f(r)] \qquad (5)$$

and results in a Morian's *I* like summary statistic $I_{mm}(r)$; this is a spatial variant of the classical Pearson correlation coefficient (Shimatani 2002) where μ_f and μ_m are the mean size of female and male dwarf palms, respectively. $I_{mm}(r)$ is normalized by $\sigma_f\sigma_m$ where σ_f^2 and σ_m^2 are the variances of the sizes of females and males, respectively.

To test if male and female dwarf palms show nonrandom spatial correlations of their sizes, we contrasted the observed mark correlation functions to a null model that randomly shuffled the sizes within the female subpopulation and the male subpopulation, thus conserving the sex specific size structure (Wiegand et al. 2013).

In all three analyses we used 199 Monte Carlo simulations of the point processes and null models for construction of simulation envelopes, being the 5th highest and 5th lowest values of the summary statistic of the simulated patterns. If the observed summary statistic was inside the simulation envelopes we considered the point process to satisfyingly describe the data. For all point pattern analyses, we used software *Programita* (Wiegand and Moloney 2014) which can be accessed at www.programita.org.

RESULTS

Dwarf palm spatial pattern

Dwarf palms at the Matasgordas plot showed two critical scales of clustering. Fit of the Thomas process with one critical scale of clustering (eq. 1) over the distance interval 5 - 70m revealed approximately 18 clusters with a size $2\sigma_L$ of 37m. However, the data showed an additional small scale clustering at distances below 4m not accommodated by this cluster process, as clearly shown by the pair correlation function (Fig. 2A).

The best fit of the Thomas process with two critical scales of clustering (eq. 2) reveals approximately 19 large clusters with sizes $2\sigma_L$ = 38m, and approximately 401 small clusters with a size $2\sigma_S$ = 5.2m which are nested within the large clusters. This cluster process fits well the pair correlation function (Fig. 2E) and the *L*-function (Fig. 2F), but the

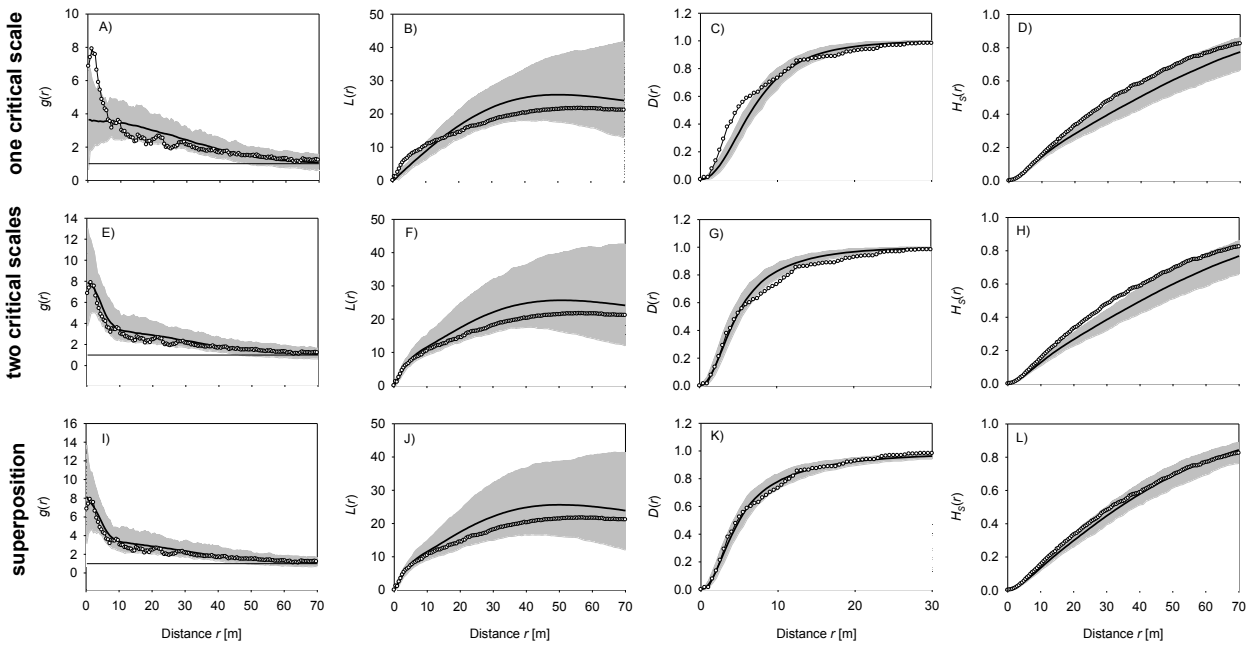

Figure 2. Cluster analysis of the Matasgordas plot. (A- D) Fit with Thomas process with one critical scale of clustering (eq. 1). (E - H) Fit with Thomas process with two critical scales of clustering (eq. 2), (I - L) Fit with superposition of a random pattern with 30 points (p_C = 0.86) with a Thomas process with two critical scales of clustering (eq. 3). Open disks: observed summary statistics, black line: expectation under the point process model, grey area: simulation envelopes being the 5th lowest and highest values of the summary statistics estimated from the 199 simulations of the fitted cluster processes.

observed nearest neighbor distribution function $D(r)$ (Fig. 2G) and the spherical contact distribution $H_S(r)$ (Fig. 2H) are outside the simulation envelopes. The observed $D(r)$ is at distances above 7m, which indicates that the observed pattern contains more isolated points than the Thomas cluster process with two critical scales of clustering. Similarly, the observed $H_s(r)$ is above the simulation envelopes (i.e., the nearest neighbor of the test points is closer than predicted), which indicates that the holes in the observed pattern are smaller than those predicted by the Thomas cluster process.

We therefore tested superposition cluster processes (eq. 3) with different numbers of random points and found that a superposition cluster process with 30 random points (i.e., p_C = 0.89) yields simultaneous agreement in all four summary statistics (Fig. 2I - L). This point process showed a factor p^2_C = 0.8 and a reduced number of large and small clusters (i.e., 15 large clusters and 318

small clusters nested within the large clusters). Thus, each large cluster comprises on average 16.3 individuals and each small cluster on average 0.77 individuals. Because the number of points per cluster follows a Poisson distribution with mean $\mu_s = 0.77$, we can estimate the expected number of empty small clusters (147), the number of clusters with one point (113), with two points (44) and more than two points (14). Thus, approximately 36% of the palms have one nearby neighbor within the same cluster and 17% two or more.

Results for the analysis of Martinazo plot were surprisingly similar to those of Matasgordas. Fit with the Thomas process with one critical scale of clustering for distances of 3-70m revealed approximately 10 large clusters with a cluster size of $2\sigma L = 44$m, but there was again a signal of an additional small-scale clustering below 2m in the data (Fig. 3A). Fit with the Thomas process with two critical scales of clustering reveals approximately 11 large clusters with a size $2\sigma L = 42$m, and approximately 368 small clusters with a size $2\sigma S = 2.8$m nested within the large clusters (Fig. 3E). Again, this cluster process does not fit the nearest neighbor distribution function D(r) (Fig. 3G) and the spherical contact distribution Hs(r) (Fig. 3H). Superposition with 25 random points (i.e., pC = 0.73) yielded simultaneous agreement in all four summary statistics (Fig. 3I - L). This point process showed by factor p2C = 0.53 and a reduced number of large and small clusters (i.e., 6 large clusters and 196 small clusters nested within the large clusters). Thus, each large cluster comprises on average 11.3 individuals and each small cluster on average 0.34 individuals. The expected number of empty small clusters yields 138, the number of clusters with one point 48, with two points 8 and more than two points 1. Thus, approximately 25% of the palms have one nearby neighbor within the same cluster and 5% two or more.

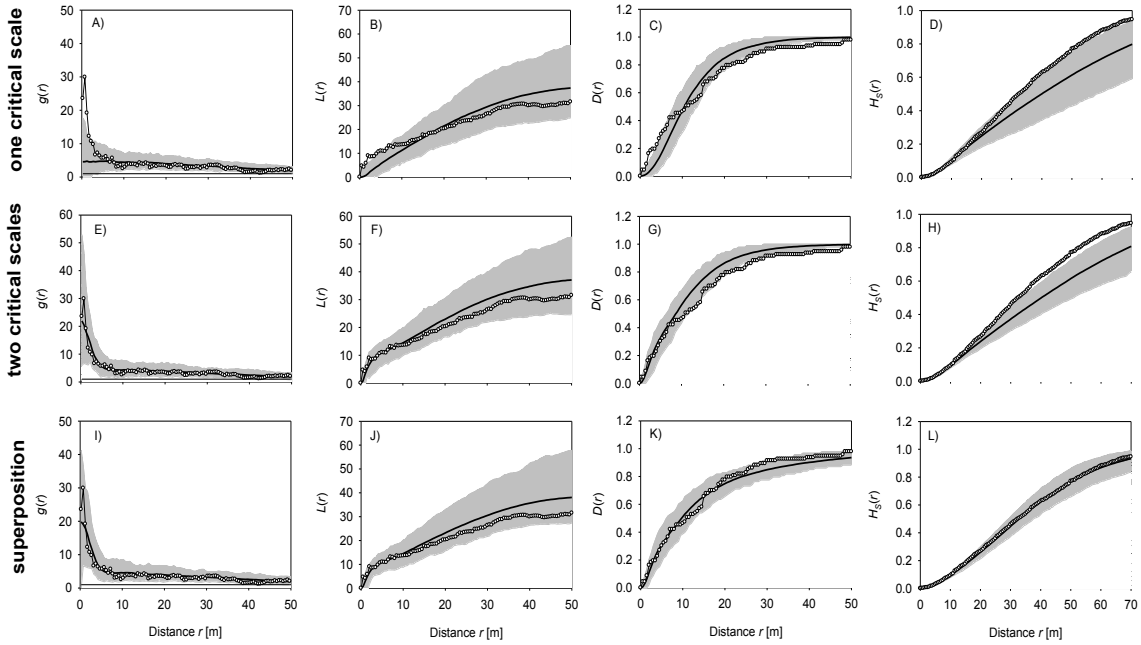

Figure 3. Same as Fig. 2, but for the Martinazo plot.

The spatial relationship between females and males

Our analysis using the random labeling null model showed that male and female dwarf palms were randomly distributed in both observational plots. Females were randomly distributed among all dwarf palms (Figs. 4A, D), males were not segregated from females (Figs. 4B, E) and the overall dwarf palm density around males and females did not differ (Figs. 4C, F).

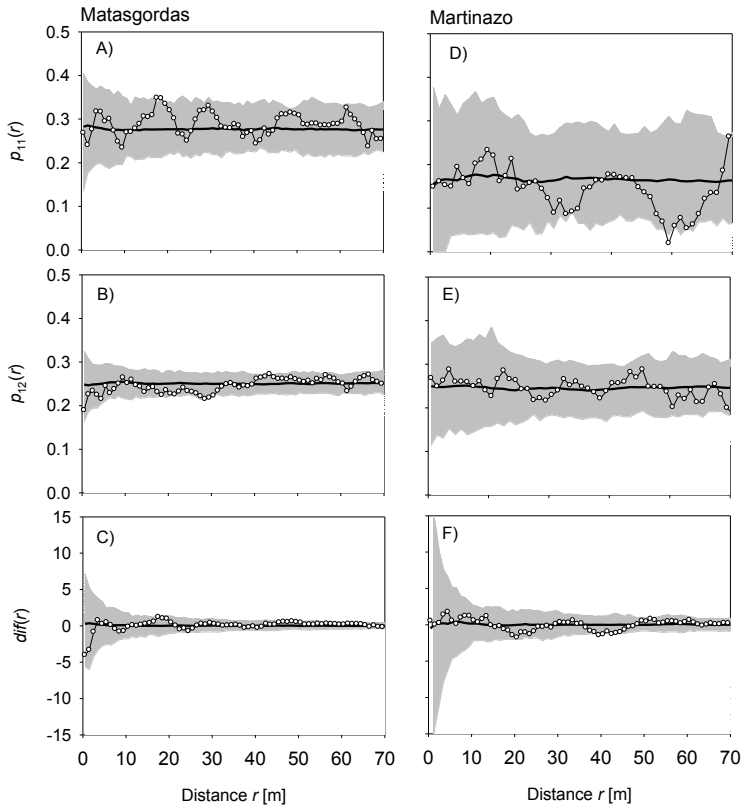

Figure 4. Results of the random labeling analysis to find out if the distribution pattern of male and female dwarf palms was random within the overall pattern of all dwarf palms. (A- C) results of the different test statistics for the Matasgordas plot, (D-F) results of the Martinazo plot. The $p_{11}(r)$ tests if females show at distance r a pattern within all dwarf palms, the $p_{12}(r)$ tests if males are at distance r spatially associated with females, conditionally on the locations of all dwarf palms, and the $dif(r)$ tests if females are surrounded at distance r by a higher dwarf palm density than males. The white circles represent the observed test statistics, the black line the expectation of the random labeling null model and the grey area represents the simulation envelopes being the fifth lowest and highest values taken from 199 simulations of the null. We used a bin of 1m and a ring width of 5m.

The spatial relationship among sizes

In relation to sizes, we found differences between the populations. At Matasgordas, individuals that have another individual located within the range of large clusters (say 7-45m) are larger than expected by the null model and individuals within the range of clustering (<45m) show spatially correlated sizes. (Figs. 5A, C). There was a very high correlation between plants sizes separated by distances below 3m (this corresponds to the small clusters), and a moderate correlation over the range of the large scale clustering. This size correlation also appeared when we analyze separately females and males (Fig. A1). In contrast, at the Martinazo plot we did not find significant values in the summary statistics, so, neither large nor small plants show a spatial

pattern. (Figs. 5B, D). This result is partly due to the smaller sample sizes, which produce substantially wider simulation envelopes.

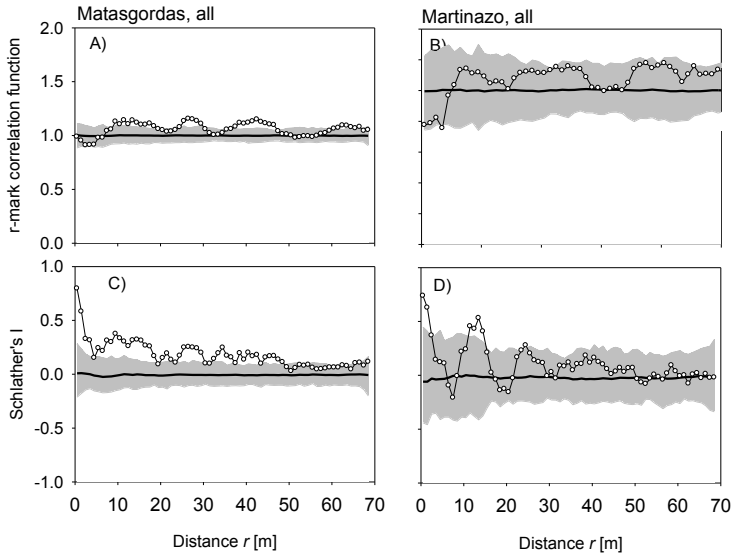

Figure 5. Results of the mark correlation analysis to find out if the sizes of female individuals were correlated with that of male individuals at distance *r*. (A - B) results for the Matasgordas site, (C - D) results of the Martinazo site. The r-mark correlation function yields the mean size of females at distance *r* of males and Schlather's I is the correlation coefficient between the sizes of all males and females separated by distance *r*. The white circles represent the observed summary statistics, the black line the expectation of the random labeling null model and the grey area represents the simulation envelopes being the fifth lowest and highest values taken from 199 simulations of the null. We used a bin of 1m and a ring width of 5m (Matasgordas) and 7m (Martinazo).

DISCUSSION

We analyzed the spatial patterns of adult dwarf palms in two areas of Doñana National Park and found clearly identifiable spatial structures. Dwarf palm spatial patterns at the two contrasting study sites were structurally similar despite substantial differences in population density. Interestingly, cluster sizes and the random distribution of the sexes were very similar between the two populations. This suggests that the same underlying mechanisms operating in populations at its colonization front generate similar spatial structures, which are then modified by different densities

In nature, clustered patterns seems to be the rule (Condit et al. 2000, Wiegand et al. 2007c), especially in plant populations dispersed by several frugivores with contrasting behaviors (e.g. *Chamaedora alternans*; Otero-Arnaiz and Oyama 2001, Fedriani et al. 2010). Fit with the Thomas process equation 3 revealed that the spatial pattern of the dwarf palm was characterized by a few large clusters (with radius of approximately 20 m) that hosted at Matasgordas and Martinazo on average 16 and 11 individuals, respectively. Additionally, we found a small-scale clustering where two or three palms shared occasionally a small cluster with radius of some 5m and 3m. Or in other words, in an average large cluster at Matasgordas with 16 palms, eight have no nearby neighbor, but 8 are arranged in groups of two. This grouping happened at Matasgordas and Martinazo for 53% and 29% of all palms, respectively. Additional to the clustered individuals, we estimated that 11% and 27% of all dwarf palms belonged to a random pattern that was independently superimposed to the clustered component pattern. Thus, we have to interpret three features of the pattern: the random palms, the small-scale aggregation and the large-scale aggregation.

Different seed disperser behaviors can impinge fruiting plant spatial patterns (Hampe et al. 2008). In the dwarf palm there is a variety of seed dispersal, from occasional dispersers like red deer (*Cervus elaphus*), to defleshers like rabbits (*O. cuniculus*) and legitimate dispersers such as badgers (*M. meles*) and red foxes (*V. vulpes*) (Revilla and Palomares 2002, Fedriani and Delibes 2011). For instances, randomly distributed dwarf palms could be explained by the long distance-dispersal carried out by the red fox, which deliver feces with seeds in a relatively scattered fashion (Fedriani et al. 2010). Also, the red deer (*C. elaphus*) and the wild boar (*Sus scrofa*), typically described as dwarf palm seed predators (Fedriani and Delibes 2011), do allow some undamaged seeds to escape and fall randomly (authors personal observation), contributing to the random plants in our plots. On the other hand, there is a hypothesis that can explain dwarf palm aggregations: the existence of complementary dispersal mechanisms. One pattern comprising the large-scale aggregations may be related to badgers seed dispersal. Interestingly, though badgers act as long-distance dispersers (Fedriani et al. 1999, Revilla and Palomares 2002) they tend to defecate dwarf palm seeds in large latrines at relatively small separation (~10 m) from the neighborhood plants (Fedriani and Wiegand 2014) fact that could increasing the size of plants patches. Besides, these large clusters were overlaid by small-scale aggregations likely related to

badgers feces that contain seeds strongly aggregated at small spatial scales and the vigorous sprouting (Fedriani and Delibes 2011). Additionally, rabbits feed on ripe fruits but they only eat the fleshy mesocarp, leaving the endocarp intact either still attached to ramets or detached and beneath mother increasing plant recruitment at very short-distances (Fedriani and Delibes 2011). Our results are similar to those found in the tropical species *Shorea congestiflora* by Wiegand et al. (2007c), with a nested double-cluster pattern explained by two independent recruitment and/or seed dispersal mechanisms. Finally, the differences between sites could be explained by disparities in dispersers' activity. In Matasgordas, the density (and average activity) of badgers is higher (2.25 tracks/km/day) than in Martinazo (1.46 tracks/km/day) (data from Monitoring Team of Natural Process of Doñana Biological Station). Fedriani and Wiegand (2014) suggest that in areas with more badger activity seed aggregation should be higher, which may increase the number of aggregated palms. Instead, the density (and average activity) of foxes is higher in Martinazo (7.45 tracks/km/day) than in Matasgordas (1.95 tracks/km/day). The fox has a scattered seed dispersal pattern (Fedriani et al. 2010) that would generate a more sparse distribution, decreasing the percentage of aggregated plants in Martinazo. Furthermore, in Martinazo the herbivore pressure by native and domestic ungulates is high (Soriguer 1983), eliminating most of dwarf palm seedlings and limiting plant aggregation.

On the other hand, our results from the random labeling agree with our hypothesis that female and male palms were randomly distributed within the study plots. Most dioecious species reflect SSS, generally correlated to environment or nutrient conditions, with males often in more nutrient-poor or stressful environments than females (Bierzychudek and Eckhart 1988, Eppley 2005, Vessella et al. 2015). Nevertheless, we found that dwarf palm females and males had a random distribution, suggesting that there was no apparent microhabitat segregation by sexes. Furthermore, apparently dwarf palm females did not experience differential germination, differential mortality or intrasexual competition, like in many other plant species with SSS (Gibson and Menges 1994, Eppley 2001, Nanami et al. 2005). The lack of differentiation between sexes in growth and survivorship was evident when we analyzed the palm sizes and there was no evident size differentiation. However, we found that plants in Matasgordas at least followed a size structure; in small and large aggregations plants had similar sizes, with a decreasing positive

autocorrelation with distance. This could be related to a facilitation process without intraspecific competition.

There are other factors that could affect the observed spatial patterns in our plots. As we described previously (see study area), both plots have been greatly affected by local human disturbances (e.g. livestock grazing), modifying and removing part of the original dwarf palm population (e.g. Camarero & Gutierrez, 1999, Thompson 2005). In the last two decades, both plots have been slowly recolonized by pyrophitic shrub vegetation composed mainly by *H. halimifolium*, *R. officinalis*, *Ulex* spp. and *Stauracanthus* spp (Soriguer 1983). This can explain the low density of dwarf palms in both plots (Matasgordas= 13.93 palms/ha; Martinazo= 4.34 palms/ha) compared with the ~400 palms/ha described in a unaltered area in a previous study by Fedriani and Delibes (2011). The persistence of patches that are small or have low conspecific density is dependent on the successful reproduction of resident plants (Debinski and Holt 2000, Groom 2001). It is possible that the dwarf palm use resident clustering as propagule sources to colonize new areas (Bolker and Pacala 1999, Colautti et al. 2006). Thus, the presence of two long-distance dispersers (badgers and foxes) enables the colonization of new areas and increases plant density. When there is significant long-range dispersal, the edge of the range may extend some distance beyond the source population, forming a colonization front sustained by recurrent immigration (Holt 1983, Pulliam 1988). For instance, this could be happen in Matasgordas, where Fedriani et al. (2010, 1999) described that badgers deliver dwarf palm seeds to habitats where this palm is absent or occur at low densities, like in the dehesa. Finally, our study plots were in a transition zone of environmental stress, between the shrubland or dehesa and the marshland. Thus, species will expand its range to fill the available habitat until the plants reach marshland or zones with high flooding regime, where deterioration of the environment limit its survivorship (Keitt et al. 2001, Lönn and Prentice 2002, Moore 2009, Drezner 2014).

Finally, populations with low-density may experience lower reproductive output than their conspecifics in large populations (e.g. Allee effect; Kéry et al. 2000, Ågren et al. 2008, Fedriani et al. 2015). Plants in small patches can be affected in their pollination regimes, being less attractive to pollinators (Fagan et al. 2014) or cause inbreeding depression because of an insufficient number of indiviuals (Ovaskainen and Hanski 2001). However, several studies have shown that some

species do no present lower fitness in marginal populations (e.g. Jump and Woodward, 2003; Kluth and Bruelheide, 2005; Samis and Eckert, 2007), suggesting that marginal populations may have other mechanisms to maintain itself. For instance, in dwarf palm contagious occurrence of large individuals of both sexes, which tend to have more flowering resources than smaller ones (Klinkhamer et al. 1987, Wesselingh et al. 1997, Méndez and Karlsson 2004), can report obvious benefits in terms of pollination success (Bessa-Gomes et al. 2003, Stehlik et al. 2006, Gascoigne et al. 2009, Fedriani and Delibes 2009a). Additionally, dwarf palm aggregations may result in potential benefits on fruit removal and dispersal (Sargent 1990, Saracco et al. 2005, Carlo and Morales 2008). In other study we have observed not only higher seed dispersal, but also higher seed predation, in more aggregated plants (Jácome et al. unpublished data). Spatial aggregation can negatively affect palm reproductive performance by attracting more seed and seedling predators (Fedriani and Delibes, 2011, Rodriguez et al. 2014). This is related with the Janzen-Connell hypothesis, according to which seeds and young plants suffer increased mortality in the neighborhood of their parent plants (Janzen 1970, Connell 1971). Several studies have shown that seedlings survival decreases with increasing density of conspecific seedlings and adults (Wills et al. 1997, Webb and Peart 1999, Wills and Condit 1999, Harms et al. 2000, Metz et al. 2010). However, Fedriani and Delibes (2011) frequently observed *C. humilis* seedlings establishing beneath fruiting palms, which suggests that the clumped pattern could "protect" seedlings from drought (e.g. Montesinos et al., 2006; Roll et al., 1997; Wied and Galen, 1998).

The SPPA gives a detailed description of the *C. humilis* spatial patterns and led us to infer some of the underlying processes generating these patterns, as well as to predict their consequences for the palm reproductive performance. This has consequences over the plant fitness: the adult plants in clusters could attract more pollinators and seed dispersers and predators, and could act as a nursery plant for its seedlings. It seems that the dwarf palm spatial patterns and dispersal strategies make this plant a successful plant for colonization of new habitats. Furthermore, is a very tolerant species adapted to high temperatures and intense droughts making a desirable species in restoration programs in the context of global change (Rodríguez et al. 2014). Clearly, to guarantee the restoration with this species, seedlings must be assembled in clumps of contrasting sizes and domestic ungulates must be removed from the area to accelerate colonization. Additional work is currently underway focusing on the effects of the dwarf spatial

patterns over pollination and seed dispersal success, predation and seedling survivorship. This data are important to know the colonization ability of the dwarf palm, and if necessary the management strategies to recover plant populations.

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APPENDIX

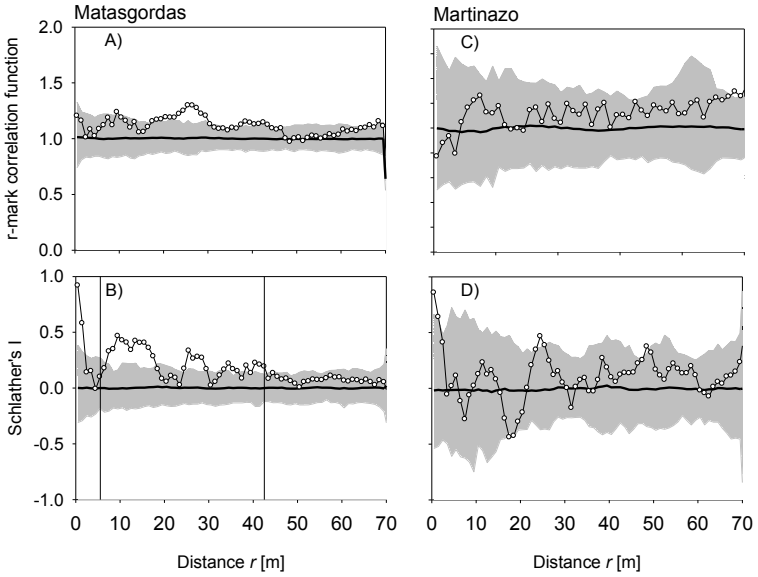

Figure A1. Same as Fig. 5, but for the pattern of females and males. Individuals that have another individual located within the range of large clusters (say 7-45m) show are larger than expected by the null model and individuals within the range of clustering (<45m) show spatially correlated sizes. All summary statistics in Matasgordas are significant. The ring width was 5m for Matasgordas and 7m for Martinazo.

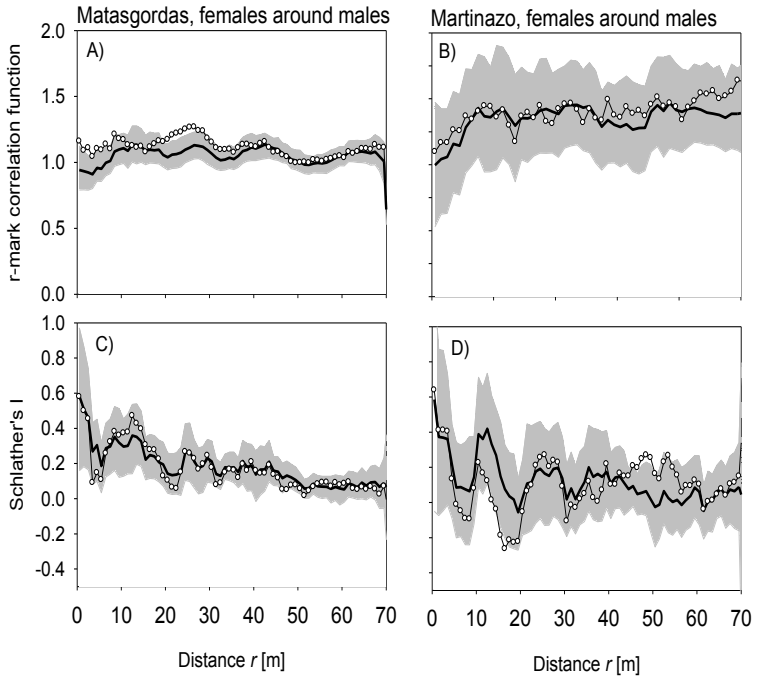

Figure A2. Same as Fig. 5, but using the random labeling null model that maintains the sizes of the individuals but randomly shuffles their label "male" and "female". The ring width was 7m for Matasgordas and 9m for Martinazo. Females at distance *r* away from males tend to be slightly larger than expected at the Matasgordas plot.

CAPÍTULO 2:

SPATIO-TEMPORAL ARRANGEMENT OF *CHAMAEROPS HUMILIS* INFLORESCENCES AND OCCUPANCY PATTERNS BY ITS NURSERY POLLINATOR, *DERELOMUS CHAMAEROPSIS*

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RESUMEN

Los sistemas de nursery pollination son sistemas altamente especializados donde los polinizadores se reproducen en las estructuras reproductivas de las plantas para completar su ciclo de vida. Desde el punto de vista de los polinizadores, la explotación de los recursos para el buen desarrollo de su descendencia es altamente dependiente del sexo de la inflorescencia y los patrones espacio-temporales de floración. En esta investigación se estudió cómo la sincronía de floración, el sexo y la distribución espacial del *Chamaerops humilis*, se relacionan con la probabilidad de ocupación y abundancia de larvas del gorgojo *Derelomus chamaeropsis*. Se analizó mediante modelos GLMM la relación entre la presencia-abundancia de larvas de gorgojo con el sexo, número de flores y la sincronía de floración. Mientras que los patrones espaciales de ocupación y abundancia de larvas del gorgojo se analizaron mediante las técnicas del el análisis espacial de patrones de puntos; correlación de marca y etiquetado aleatorio. Encontramos que las inflorescencias masculinas fueron las inflorescencias con mayor presencia-abundancia de larvas de gorgojo. Sin embargo, encontramos que aproximadamente el 30% de las inflorescencia femeninas eran también capaces de mantener las larvas. Además, encontramos que las plantas altamente sincrónicas con pocas inflorescencias fueron más propensas a contener larvas, quizás porque estas plantas evitan un efecto de dilución ovoposición.Finalmente, aunque las plantas de *C. humilis* están espacialmente agregadas no detectamos ningún patrón espacial de la ocupación de larvas. Estos resultados son el primer intento de relacionar los patrones espacio-temporales de las plantas hospederas con la presencia y abundancia de su polinizador.

ABSTRACT

Nursery pollination is a highly specialized system where pollinators reproduce inside plant reproductive structures. The successful development of the pollinator offspring is highly dependent on the inflorescence sex, as plants defend female reproductive structures, and on the spatio-temporal flowering pattern. In this research we studied how the sex, flowering synchrony and spatial distribution of the dwarf palm *Chamaerops humilis* relate with the occupancy probability by the larvae of its pollinator, the weevil *Derelomus chamaeropsis*. We collected larvae in the inflorescences during three seasons. Relations between the presence-abundance of weevil larvae and the sex, number of flowers and flowering synchrony were analyzed with GLMM models. Instead, spatial patterns of weevil larvae were described using correlation analysis and random brand labeling techniques within the spatial analysis of point patterns. We found that male inflorescences were those with more presence-abundance of weevil larvae, although approximately 30% of the females were capable to hold larvae. Also, plants highly synchronic with low number of inflorescences were more prone to hold larvae, perhaps because these plants avoid an oviposit ion dilution effect. Finally, although *C. humilis* followed a clumped pattern we were unable to detect any spatial pattern of larvae occupation. These results are the first attempt to relate any plant temporal and spatial patterns to the presence and abundance of its nursery pollinator.

INTRODUCTION

Pollination by animals is a key ecosystem service for the maintenance of both wild plant communities (Aguilar et al., 2006; Ashman et al., 2004) and agricultural productivity (Klein et al., 2007; Ricketts et al., 2008). Up to 87 % of all flowering plant species rely on this mutualistic interaction, involving several groups of insects and small vertebrates (Buchmann and Nabhan, 1996; Harder and Barrett, 2006; Richards, 1986). In this interaction, the plants offer a reward (pollen, nectar, refugee or oviposition sites) in exchange for pollination services. Resource availability for pollinators (usually, open flowers) markedly varies, both in time and space, determining their patterns of activity and efficiency (Anker, 1990; Eckhart, 1991; Fedriani et al., 2015; Suzuki et al., 2003).

In general, pollinators are attracted to a given plant or group of plants only after a certain threshold density of flowers in bloom (Fagan et al., 2014; Marquis, 1988; Rathcke, 1983). Thus, one important trait is the flowering synchrony of individuals (i.e. in relation to the population flowering peaks) and the other the number of flowers per plant (Augspurger, 1981; Melampy, 1987). Besides, the level of plant aggregation across the landscape determines the successful encounter of the host plant by its pollinators, large and well connected plant aggregations being more attractive to pollinators than isolated ones (Aizen and Vázquez, 2006; Fedriani et al., 2015). For instance, in species like *Cyprepedium japonicum* (Orchidaceae) and *Pyrus bourgaeana* (Rosaceae) flowering individuals growing in aggregations generally had higher degree of flowering synchrony and were more attractive to pollinators (Fedriani et al., 2015; Sun et al., 2009).

The effect of the spatio-temporal flowering pattern on pollination success has been studied in many pollination systems (Dupont et al., 2009; Eckhart, 1991; Price et al., 2005; Traveset and Sáez, 1997). However, few studies (i. e. Aker, 1982) address this interesting topic in systems where the pollinators not only feed on the floral structures but also used them as oviposition sites. The so-called nursery pollination system involves coevolution of two species implying a variety of costs and benefits (see Dufaÿ and Anstett, 2003). In this system, plants provide to the pollinator with egg-laying sites (e.g. seeds, ovaries, inflorescences) and resources for larvae development in

exchange of pollination services (Anstett et al., 1997; Cook and Rasplus, 2003; Dufaÿ and Anstett, 2003; Herre et al., 2008). In dioecious species, the interaction involves sex-specific components that play an important role, conditioning the egg-laying site selection, larvae development and the reproductive plant costs. For instance, when pollinators mainly oviposit on male inflorescences, female inflorescences could suffer pollinator limitation but, low ovaries predation (Anstett, 2001; Feil, 1992; Norstog and Fawcett, 1989). Because of this, plants have often developed methods to reduce the cost of rearing pollinator larvae in female inflorescences; thus, female plants are partially pollinated by deceit, as they do not pay a reward for the service (i.e. they are cheaters) (e.g. Dufay and Anstett 2004). Simultaneously, plants also developed strategies to attract pollinators to the cheater female inflorescences, such as intersexual odor mimicry (Dötterl and Jürgens, 2005; Dufaÿ et al., 2004), in order to ensure pollination. Moreover, in dioecious species the arrange and availability of opposite sex individuals within a patch can strongly impinge on the reproductive success and pollinators spatial distribution (Davis et al., 2004; Gascoigne et al., 2009; Groom, 1998; Kunin, 1993).

In this study we analyzed how the flowering synchrony, sex and spatial distribution of the plants relate with the occupancy probability by larvae of a nursery pollinator. To this end, we investigated the interaction between the Mediterranean dwarf palm *Chamaerops humilis* and the larvae of its weevil pollinator *Derelomus chamaeropsis*. In this specialized mutualism, once pollinating weevils have found a plant, they typically stay until the end of its anthesis, finding shelter, egg-laying sites and food within the inflorescences (Dufaÿ et al., 2004). When a plant reaches the end of anthesis, weevils leave it to search for a new host plant. Oviposition occurs mostly, but not only, within the male inflorescences during spring and summer (Anstett, 1999; Dufaÿ and Anstett, 2003; Dufaÿ et al., 2004). Larvae development happens during autumn and winter and apparently only in rachises of old male inflorescences (Dufay and Anstett 2004). There are no studies trying to identify how the spatial and temporal patterns of inflorescences affect the presence and abundance of a nursery pollinator. We explored different hypothesis: 1) Because the cost for the plant would be lower, weevil larvae development should occur almost exclusively in male inflorescences; Dufaÿ and Anstett (2004) postulated in experimental conditions this cheater role for females, suggesting they have mechanisms to destroy the larvae; thus, we tested if larvae development also happens only in male inflorescences in field conditions. 2) Because highly

synchronic plants tend to be more attractive to pollinators (Cunningham, 2000), we expected that synchronic dwarf palms will have a positive relation with the presence and abundance of weevil larvae. 3) The number of inflorescences has been also positively related to the number of pollinators (Knight et al., 2005), thus, we expected that the number of inflorescences in each plant will impinge positively the presence and abundance of weevil larvae. 4) High-density neighborhoods can attract more pollinators (Aguilar et al., 2006), thus, we propose that the study site with a large flowering dwarf palm density will have more weevil larvae. 5) Because a coupled spatial pattern has been described for dwarf palms at the study area (Jácome-Flores et al., unpublished), we predict that larvae presence and abundance will follow the same pattern at similar scales. 6) We expect that the interaction between synchrony and number of inflorescences will have a stronger effect than these variables by their self.

MATERIAL AND METHODS

Study species and area

The study was carried out in the Doñana National Park (510 km^2; 37º9' N, 6º26' W) located on the right bank of the Guadalquivir estuary in southwestern Spain. The weather is characterized by average annual temperatures between 15.37 and 18.67 ° C (mean = 16.91 ± 1.06 º C; n =35; period 1978-2013). Similarly, the annual rainfall varied during this period between 170 and 1028 mm (mean=542.6 mm ± 12.02 mm; data from Monitoring Team of Natural Process of Doñana Biological Station; http://www-rbd.ebd.csic.es/Seguimiento/mediofisico.htm). Between November-December of 2011 we selected and delimitated two observational study sites within Doñana area, called Matasgordas and Martinazo (Fig. 1), where we identified and georeferenced (with a submetric GPS) all breeding *C. humilis* individuals (a total of 399). The vegetation and physiographic characteristics differ between study sites.

The Matasgordas site is occupied by a dehesa, which includes some areas prone to flooding and is limited in the south by a marshland (Fig. 1). The dehesa habitat (~ 300 ha) has a continuum of grasslands with open tree stratum of *Q. suber*, *O. europaea* var. *sylvestris*

and *Fraximus angustifolia* with no, or only sparse, understory of Mediterranean scrubs (Fedriani et al., 2010). In this site we delimited a study site of 22.1 ha with 294 marked individuals (167 females, 127 males) with a density of 13.03 ind/ha. The Martinazo site is covered by a dense Mediterranean scrubland dominated by *Halimium halimifolium*, *Rosmarinus officinalis*, *Ulex* spp. and *Stauracanthus* spp. The area is composed mainly by a pyrophitic scrub (*Ulex* spp., *Stauracanthus* spp. and *C. humilis*) (Granados et al., 1988). Within this locality we delimitated a study site of 20.93 ha with 91 adult individuals (42 females, 49 males) with a density of 4.35 ind/ha.

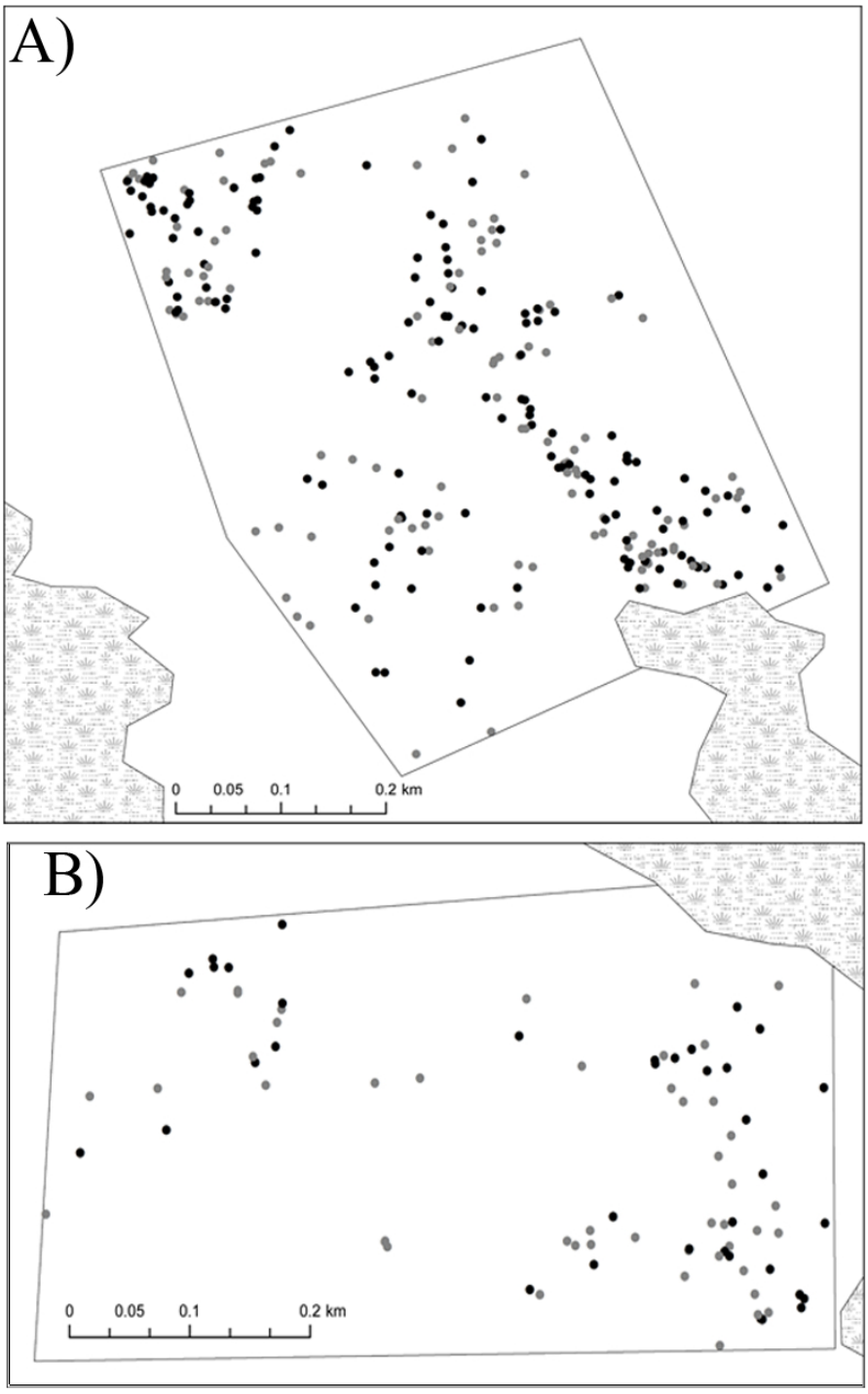

Figure 1. . The two study sites limited by the marshland (grass pattern) within the Doñana National Park area. The study sites (A) Matasgordas and (B) Martinazo with the georeferenced plants: the points in black represent female plants, and the gray points the males..

Data collection

Identification and quantification of D. chamaeropsis larvae

In December 2011, 2012 and 2013 for each reproductive dwarf palm we registered its sex and collected randomly two inflorescences of the previous flowering season (overall, N = 1063 inflorescences). In the lab, with a pocketknife, we carefully dissected the old rachis making a longitudinal cut along the base through all the inflorescence ramifications avoiding the accidental destruction of larvae. Finally, we extracted, identified and counted the number of *D. chamaeropsis* larvae (overall, n = 5986 larvae; Figure 2).

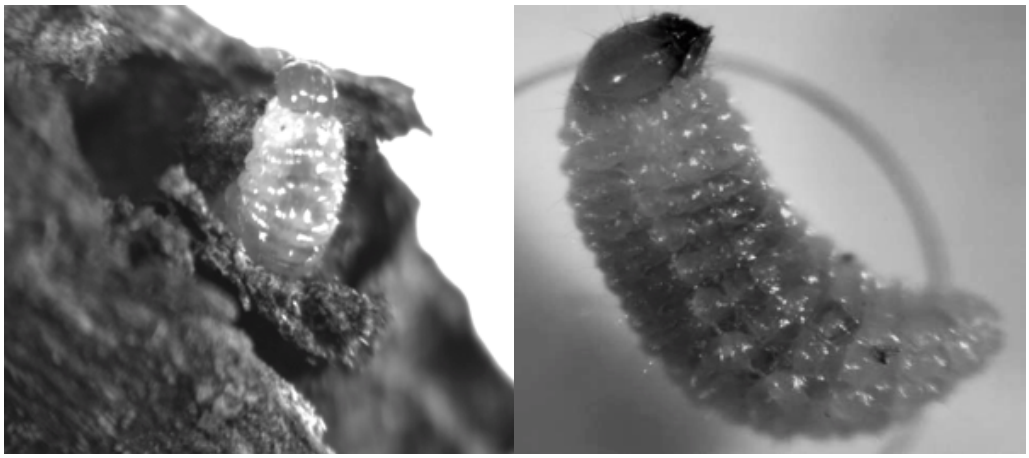

Figure 2. Images of *D. chamaeropsis* larvae emerging from an old inflorescence and then placed for identification.

Dwarf palm flowering traits

During the flowering season (February-May) of 2013 we counted all the inflorescences produced by each reproductive individual during the flowering season. Also, every 12 days we counted the number of inflorescences in anthesis and calculated a flowering synchrony index for each individual in relation to all the others, using the method of Augspurger (1983):

$$Xi=\left(\frac{1}{n\text{-}1}\right)\left(\frac{1}{fi}\right)\sum_{j=i}^{n} e_{j\neq i}$$

Where $e_{j\neq i}$ is the number of days that individuals *i* and *j* overlap in their flowering; f_i is the total number of days individual *i* is flowering; and n is the number of individuals in the sample. When the flowering time of an individual overlaps completely with all other individuals X = 1. When there is no overlap in an individual's flowering time X = 0. A measure of the overall synchrony of the population is gained by averaging the individual synchronies.

Early periods of low flower production are unlikely to contribute much to the reproductive output of an individual plant. However, larvae only develop in dry, dead rachides and the early produced inflorescences could provide suitable egg-laying sites for the *D. chamaeropsis*.

Larvae occupation spatial patterns

In a previous work, Jácome-Flores et al. (unpublished) demonstrate through Spatial Point Pattern Analysis (SPPA) (Wiegand et al., 2006) that the dwarf palms in our study sites have a strongly contagious pattern, characterized by large clusters (radius<40 m) composed by small aggregations (radius<5.2 m). To relate the spatial larvae occupancy patterns with the described dwarf palm spatial patterns, we assigned the data of larvae presence and abundance in each georeferenced plant and perform two techniques of the SPPA: random labeling and mark correlation function

To examine the distribution pattern of presence/absence of larvae in plants is explained or correlated with their location we used the random labeling null model to represent absence of spatial structure (Wiegand and Moloney, 2014). The qualitative mark is of type 1 if the dwarf palm had weevil larvae, and 2 if not. A mark connection function *pij*(*r*) gives the conditional probability that, from two dwarf palms that are separated by distance *r*, the first is type *i* and the second type *j* (i.e., *i* =1 or 2; Illian et al., 2008).

$$p_{ij}(r) = p_i\, p_j\, \frac{g_{ij}(r)}{g_{i+j,\,i+j}(r)}$$

where the $g_{ij}(r)$ are partial (or bivariate) pair correlation functions that quantify the relative density of type *j* dwarf palms around type *i* points (Wiegand and Moloney, 2004), the $g_{i+j,i+j}(r)$ is the pair correlation function of the unmarked pattern (i.e., dwarf palms with and without larvae), and p_i is the proportion of type *i* dwarf palms among all dwarf palms. If dwarf palms with larvae are a random sample of all dwarf palms, we expect $p_{ij}(r) = p_i p_j$. If dwarf palms with larvae are aggregated within all dwarf palms, we find $p_{11}(r) > p_1\, p_1$, and if dwarf palms with and without larvae are spatially segregated, we find $p_{12}(r) < p_1\, p_2$. To test if dwarf palms containing larvae are preferably located in areas of overall high density of dwarf palms (e.g., high density clusters), we used the test statistic $g_{1,1+2}(r) - g_{2,1+2}(r)$ (Jacquemyn et al., 2010). This test statistic compares the density of dwarf palms (i.e., 1 + 2) around dwarf palms with larvae (i.e., type 1) with the density of dwarf palms (i.e., 1 + 2) around dwarf palms without larvae (i.e., type 2). The expected value of this test statistic is zero under random labeling, but if dwarf palms containing larvae would occur preferably in clusters of dwarf palms.

We used the mark-correlation functions to test if the number of larvae in each dwarf palm was correlated, conditionally to the spatial location (Illian et al., 2008; Law et al., 2009). Our data comprise for each individual of *C. humilis* the coordinates, its sex (male or female) and the abundance of larvae (AL). The bivariate mark correlation functions consider all pairs of male and female dwarf palms (with index i, j and their marks m_i and m_j, respectively) that are located at distance *r* and estimate the mean of a test function $t(m_i, m_j)$ over these pairs, and divide it by the expectation of the test function taken over all pairs, irrespective on their spatial separation. The mark correlation function that uses the AL of the female j as test function

$$t(m_i, m_j) = m_j, \qquad (2)$$

yields the conditional mean AL $\mu_f(r)$ of females that have males dwarf palms at distance r. This test function yields the so-called r-mark correlation function $k_{.m}(r) = \mu_f(r)/\mu_f$ (Illian et al. 2008) where μ_f is the mean AL of females. Thus, $k_{.m}(r) > 1$ indicates that females which have males at distance r are on average more larvae than expected. Conversely, $k_{m.}(r) < 1$ indicates that females that have males at distance r have on average lesser larvae than expected.

We are also interested in the correlation between the AL of male and female dwarf palms that are distance r apart. The appropriate test function for this purpose was proposed by Schlather et al. (2004):

$$t(r, m_i, m_j) = [m_i - \mu_m(r)][(m_j - \mu_f(r)] \qquad (3)$$

and results in a Morian's I like summary statistic $I_{mm}(r)$, a spatial variant of the classical Pearson correlation coefficient (Shimatani 2002) where μ_f and μ_m are the mean AL of female and male dwarf palms, respectively. $I_{mm}(r)$ is normalized by $\sigma_f\sigma_m$ where σ_f^2 and σ_m^2 are the variances of the AL of females and males, respectively. In the case of a positive spatial correlation between the AL of male and female dwarf palms at distance r, dwarf palms with higher (or lower) than average AL tend to be located at distance r. Conversely, in the case of negative correlations between the AL of male and female dwarf palms at distance r, the males of the pairs show below average and females above average values of AL (or the males above average and females below average values of AL).

In all three analyses we used 199 Monte Carlo simulations of the point processes and null models for construction of simulation envelopes, being the 5th highest and 5th lowest values of the summary statistic of the simulated patterns. If the observed summary statistic was inside the simulation envelopes we considered the point process to satisfyingly describe the data. For all point pattern analyses, we used software *Programita* (Wiegand and Moloney, 2014) which can be accessed at www.programita.org.

Statistical analysis

For the statistical analysis of the larvae presence and abundance we performed Generalized Linear Mixed Models (GLMM) through the GLIMMIX procedure (SAS; Littell et al. 2006). Specifically, the presence of larvae was analyzed using a binomial GLMM with a *logit* link function and larvae abundance using a *Poisson* GLMM with a *log* link function. First, we quantified the effect of sex and study site over the larvae presence and abundance over the period 2011-2013. Study site (SS) and plant sex (S), as well as their second-order interaction were included as fixed factors. Second, we analyzed the larvae presence and abundance incorporating flowering synchrony and number of inflorescences obtained in 2013. Study site, plant sex, synchrony (Sn) and number of inflorescences (Ni) as well as their second-order interaction were included as fixed factors. For this analysis we established several competing models with different variable combination and selected the best model based on Akaike Information Criterion (AIC) as proposed by Burnham and Anderson (2002).

For all GLMMs individual plant (nested within study site) and inflorescence (nested within individual) were included as random factors to control for their potential effects. When appropriate, year was also specified in the models as random factor (Bennington and Thayne, 1994). We calculated the adjusted means and standard errors using the LSMEANS statement and were back-transformed using the appropriate Taylor's series approach (Littell et al., 2006). In the case of significant interactions, we tested for the effect of a given factor at the different levels of the other factor (i.e. tests of slices). We performed using the SLICE option in the LSMEANS statement of the MIXED procedure (Littell et al., 2006).

RESULTS

Larvae presence and abundance related to plant sex and study site

The effect of sex and study site on the presence and number of larvae was evaluated for the seasons 2011, 2012, and 2013. For the presence model, we found significant differences between the sexes ($F_{1, 2074}$= 123.23, P<0.0001). Larvae occurred more often in male (0.83) than in female (0.29) plants (Figure 3A). Besides, we found significant differences ($F_{1, 2074}$= 13.45, P=0.0003) between study sites, with Matasgordas having a lower probability of hosting larvae (0.48) than Martinazo (0.68). However, we did not find significant differences ($F_{1, 2074}$= 0.06, P=0.8052) in the second order interaction between sex and the study site.

In the abundance model the interaction between sex and study site, was significant ($F_{1, 293}$= 15.09, P=0.0001). Although, in both populations the male plants had more larvae than the female plants, the difference was greater in Matasgordas (Figure 3B). Tests of slices indicated that in both locations Matasgordas ($F_{1, 293}$= 120.07, P<0.0001) and Martinazo ($F_{1, 293}$=8.04, P<0.0049) the differences between males and females are significant. Moreover, in Matasgordas the male plants have significantly more larvae that the males in Martinazo (test of slices $F_{1, 293}$=2.51, P<0.0001). Instead, there were no significant differences (test of slices $F_{1, 293}$=22.03, P<0.1139) between the larvae abundance at the female plants of both study sites.

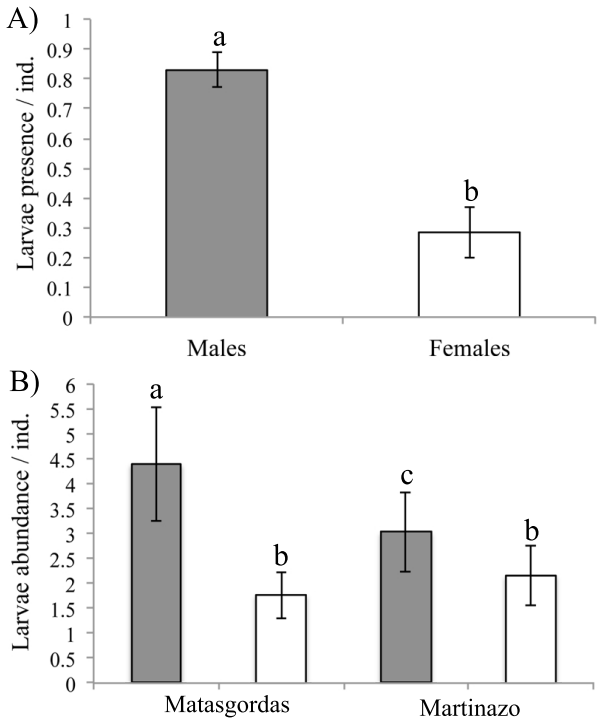

Figure 3. (A) Larvae presence in each sex and (B) larvae average abundance by sex in each study site, where the gray bars represent the male plants; the white bars represent the females.Different letters means significant differences between bars.

Larvae presence and abundance incorporating inflorescences traits

For the data of 2013 season we incorporated the measures of flowering synchrony and number of inflorescences. The production of female and male inflorescences peaked around April 14t-16th in both populations. In April the 97 % of the female plants and 94 % of the male plants had reached anthesis. The general trend in flowering was that plants showed a gradual increase in flower production since February to April with an abrupt decreasing on May, following a unimodal distribution (Fig. 3). The total flowering time was 121 days in Martinazo and 109 days in Matasgordas. Overall, we found that in general *C. humilis* showed a medium population synchrony (< 0.68), ranging from 0.18 to 0.92. However, through the GLMM performed for the synchrony index corrected for the effects of random factors, we found significant differences between sexes ($F_{1, 10}$ =21.69, p=0.0009), the males being on average more synchronic (0.75 ± 0.01) than the females (0.67 ± 0.02). However, we did not find significant differences ($F_{1, 10}$=3.93, p=0.08) between the two study sites. For the number of inflorescences, our GLMM did not reveal

any significant differences in the mean number of inflorescences produced by males (40.84 ± 2.37) and females (38.99 ± 2.29; $F_{1,1}$ =0.32 p = 0.673); we neither found significant differences between the study sites ($F_{1,1}$=37.69, p=0.1028).

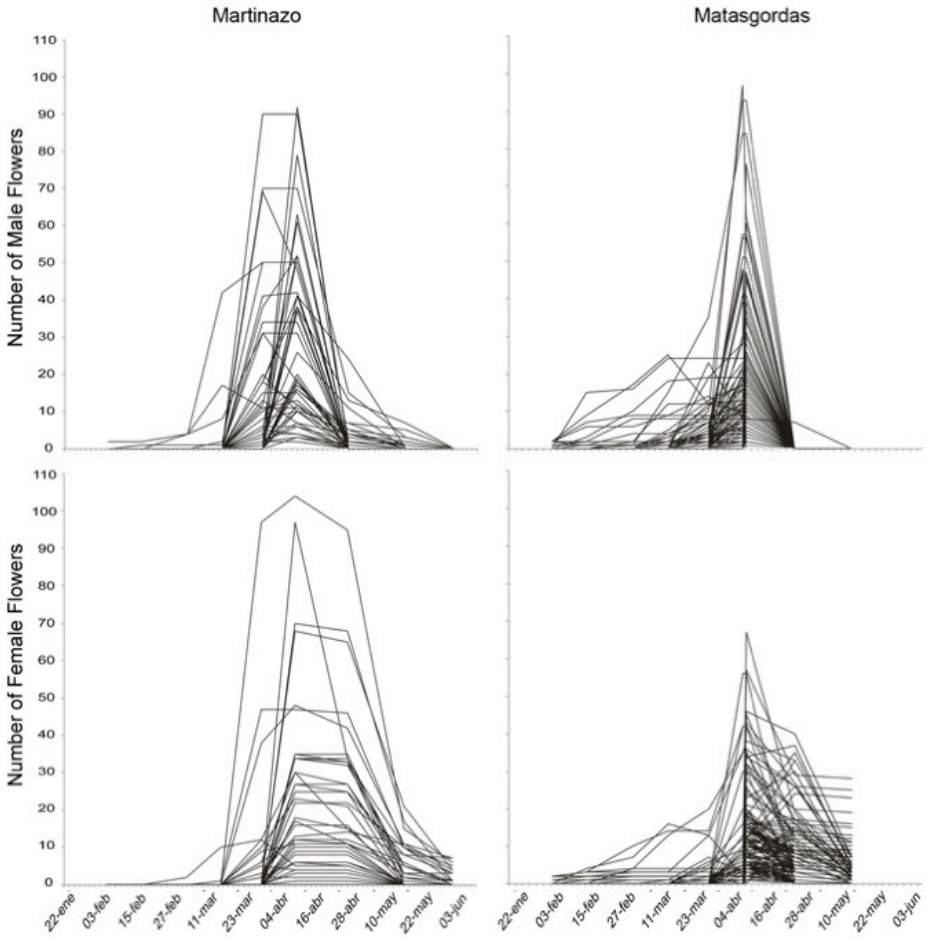

Figure 4. Flowering phenology patterns for all male and female plants in Martinazo (Males= 50, Females= 41) and Matasgordas (Males=145, Females=169).

Incorporating the measures of flowering synchrony and number of inflorescences we found that according to the ΔAICc<2 model selection, two best-supported models (8 and 10; Appendix, Table 1) for, the presence of larvae. However, the most parsimonious model was the model without the interaction between sex and flowering synchrony, where the differences in sex flowering synchrony did not have effect over the larvae presence. Instead, for the abundance models we found three good models (5, 7 and 9; Table 2) where the interaction between sex and flowering synchrony seems to be constant in all models. We selected the most parsimonious model

that in this case was the model-5 with only one interaction. The results of selected models were used to evaluate the effect of each variable (Appendix, Table 2).

For the presence model we found relevant effects of all main factors except flower synchrony (Appendix, Table 2). Specifically, males were more prone to hold larvae (0.80 ± 0.03) than the females (0.33 ± 0.04). Study site had an effect over the larvae presences where the plants in the high-density study site had lower probability of holding larvae (Matasgordas= 0.52) compared with the low-density study site (Martinazo= 0.65). In the first significant interaction (sex and number of inflorescences) we found that the dwarf palms with more probability of having larvae were the male plants (estimate=-0.97) with low number of inflorescences; the same negative relation occurs in females with low number of inflorescences but intensity of this interaction was higher (estimate= -0.114). The second interaction (number of inflorescences and flowering synchrony) was related with an increased probability of having larvae not only in the plants that are more synchronic, but also, unexpectedly in those with low number of inflorescences.

For the abundance model, the sex was again a variable with an important effect, along with the number of inflorescences and study site (Table 2). We found that male plants have more larvae (8.45 ± 0.53) than females (3.96 ± 0.36). This means that male plants not only are more prone to have larvae but to have in it in greater numbers. The synchrony of the dwarf palms has a significant positive effect (estimate=0.72) over abundance. Furthermore, we found that dwarf palms in Matasgordas study site have in average more larvae (6.4) than dwarf palms in Martinazo (5.2). Finally, interaction among synchrony and sex has an important effect, most synchronic female and male plants having in average more larvae, but the effect is stronger in male dwarf palms (estimate males=1.75, females=1.45).

Larvae presence and abundance spatial patterns

Random labeling analysis showed that the presence of larvae in dwarf palms did not depend on the degree of dwarf palm aggregation or spatial pattern, as we can corroborate for the two study

sites in the two years that the patterns were inside the envelopes (Figure 5). The results of $p11(r)$ (Figure 5), indicated that the probability that two dwarf palms have larvae in its inflorescences separated by any distance did not significantly deviated from a expectation based on a random labeling model. The bivariate function $p12(r)$, showed that dwarf palms with and without larvae are randomly distributed. Finally the test of $g_{1,1+2}(r)$ - $g_{2,1+2}(r)$ (Difference), showed that the dwarf palms with larvae was not related to the plants aggregations. Thus, larvae occupation spatial patterns did not follow an aggregation pattern like the dwarf palm spatial pattern.

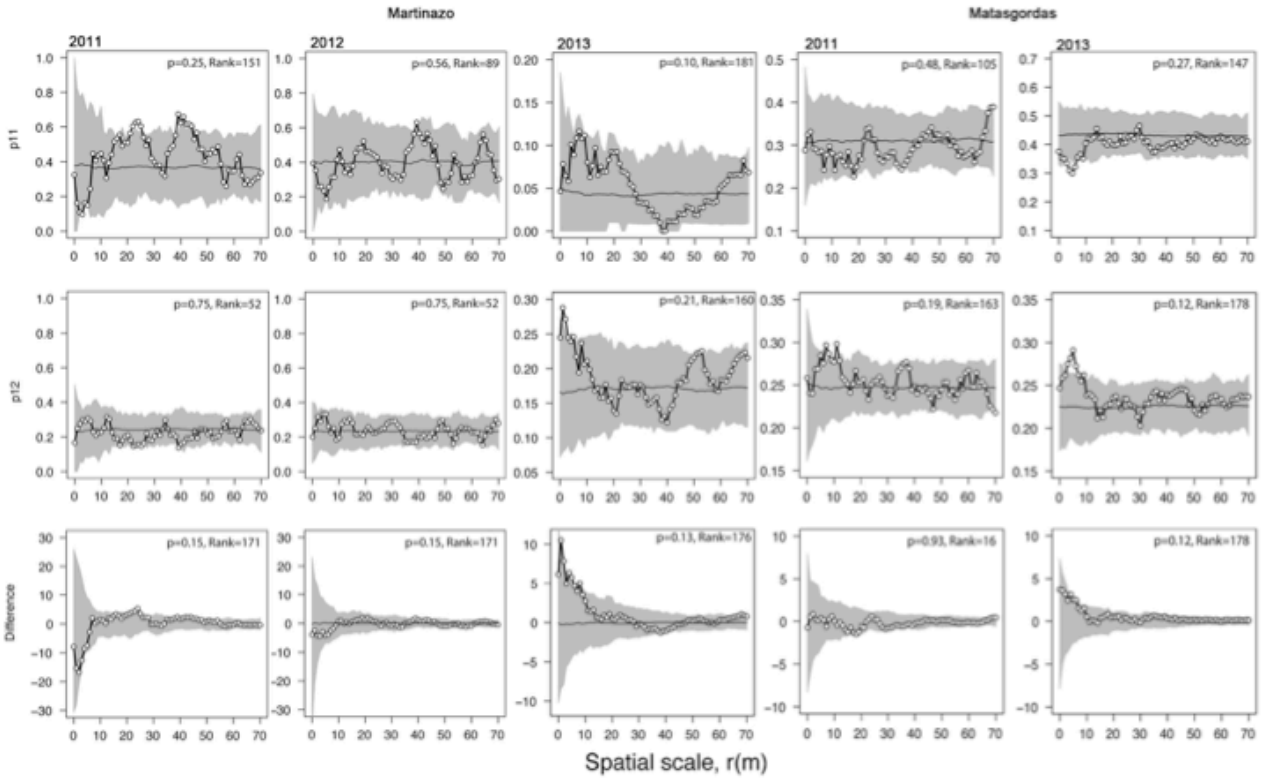

Figure 5. Random labeling for the larvae presence and absence in all the plants. $p11(r)$: tests the conditional probability that, from two dwarf palms that are separated by distance r, both are type 1. $p12(r)$: gives the conditional probability that, from two dwarf palms that are separated by distance r, the first is type 1 (i.e., with larvae) and the second is type 2 (i.e., without larvae). Difference: compares the density of dwarf palms (i.e., 1+2) around dwarf palms with larvae with the density of dwarf palms around dwarf palms without larvae (i.e., type 2). The white circles represent the distribution for the used statistic. Close circles: empirical mark connection function, grey polygon: expectation under the null model, and black lines: simulation envelopes being the fifth lowest and highest values taken from 199 simulations of the null model where plants with larvae in without it where independently shuffled.

To test if the abundance of larvae in the dwarf palm depends of the location of each sex, the mark correlation function was used as statistic. There was no significant aggregation of the larvae abundance at any spatial scale. The different number of larvae in each dwarf palms is randomly

distributed in both study sites (Figure 6). The only spatial structure that we can found was for the year 2011 in Matasgordas, where we found a significant (p=0.03, rank= 196) correlation at small spatial scale (5-10 m), between the abundance of larvae in both females and males. This means that when we find females and males located between 5-10 m apart have higher number of larvae than the expected by the null model.

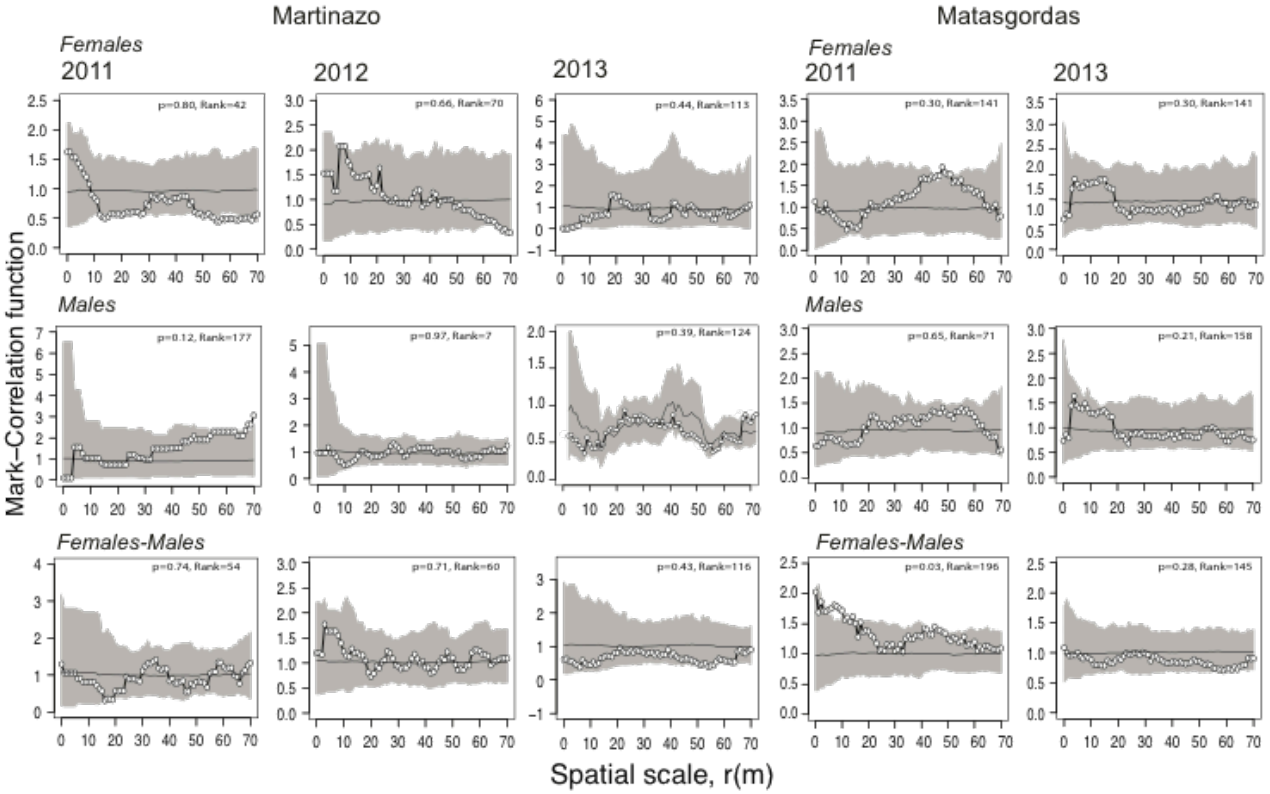

Figure 6. Univariate mark correlation function for each sex (males and females) and bivariate mark for all individuals (females-males), both used to quantify potential spatial associations of the larvae abundance in both populations in two years. The expected mark correlation function (white circles) and the corresponding simulation envelopes (grey polygon) expectation under the null model, being the fifth lowest and highest values taken from 199 simulations of the null model where both sexes where independently randomized.

DISCUSSION

In our knowledge, this is the first study that addresses the effects of flowering synchrony and spatial patterns of a plant on the presence and abundance of larvae of its nursery pollinator. At the plant level, we found a strong relation between the larvae presence/abundance and plants sex. We found that the synchrony and number of inflorescences are relevant variables for the weevil larvae abundance and presence. However, the effects were not always consistent with our hypothesis. At population level, we found that for the data of 2011-2013 the study site had no effect over larvae presence and abundance, except for the differences on larvae abundance in the male inflorescences in each site. Instead, when we incorporated flowering synchrony and number of inflorescence in 2013 study sites differ on larvae presence and abundance but the differences were unexpected. Finally, we were unable to detect a spatial pattern of the larvae occupancy.

Perhaps one of the most interesting findings is related to the sex of plants. Leaves of both sexes produce volatile compounds during the flowering season (Dufaÿ et al., 2003) that attract weevils and ensure the pollen transportation and fertilization. We found that male plants had higher probability of hosting larvae as well as higher numbers of them. According to Dufaÿ and Anstett (2004) the weevil larvae only develop within the male inflorescences, with advantages on the reproductive success of the dwarf palm, because it does not affect pollen production nor fruit development (Anstett, 2001; Feil, 1992; Norstog and Fawcett, 1989). Furthermore, these authors suggested that weevil larvae cannot develop on female inflorescences due to a defense mechanism during processes associated with fruit development. In this three years study in the field we found that almost 30 % of the female dwarf palms were holding weevil larvae. However, we observed that in general the female inflorescences with larvae were those with few fruits or even without fruits, it seems to be a negative relation between the number of fruits per inflorescence and the larvae presence. This suggests that these inflorescences could have a reduced defensive capacity and allowed the larval development (e.g. low defensive resin production, authors personal observation). An alternative explanation is that females sacrifice a fraction of their inflorescences to nourish pollinator larvae, increasing the probabilities of pollination for the adjacent inflorescences. These findings suggest that weevils reproduce in female structures, although at a

lower proportion than in males, making the first ones only less rewarding plants, and not non-rewarding-cheater plants as suggested by Dufaÿ and Anstett (2004).

When we incorporated the flowering traits synchrony and number of inflorescences to describe the larvae presence and abundance, we found different effects. Specifically, we found that synchronous dwarf palms held more larvae than asynchronous ones. Many studies showed that highly synchronic flowering plants are more attractive to pollinators (Ashman, 2005; Castilla et al., 2011; Eckhart, 1991). In our system, this pattern could be related to a synchrony between two events: weevil emergence and flowers anthesis (Dufaÿ, 2010). If a plant flowers earlier than weevils emergence, there will be less probability that a weevil oviposit in this one; on the other hand, if a plant flowers too late, there will be few available weevils because the majority already have oviposit in other plant. Of course, the effect of this variable is stronger when we analyzed its interaction with sex, due to the facts explained above. Furthermore, we found in our study sites that male dwarf palms tend to be more synchronic, and females end its anthesis after. This could have a positive effect over the oviposition and pollination of female dwarf palms. Highly synchronic males reach the end of anthesis at the same time, leaving no resource for weevils, so they have to leave these plants and travel to less -rewarding females with available inflorescences in anthesis. On the other hand, contrary to our predictions the number of inflorescences relate negatively with the presence of larvae on both sexes. In general, the number of inflorescences has been positively related to the number of pollinators (Knight et al., 2005), where pollinators are often attracted to plants where the resources are more abundant. Perhaps, plants with high number of inflorescences had lesser probability to hold weevil larvae due to a dilution effect, because weevils will oviposit in few inflorescences and many others will remain without larvae. Plants in our study sites could experience infraspecific pollinator competition, resulted from pollinator dilution among the multitude of flowering resources (Delmas et al., 2014; Fritz and Nilsson, 1994; Larson and Barrett, 2000). Finally, the interaction between synchrony and number of inflorescences had a stronger effect only for the larvae presence. We found that the dwarf palms with the highest probability of holding larvae were those with high flowering synchrony values (i. e. plant anthesis coincide with weevil emergence) and with few inflorescences, which increase the probability of oviposition.

In terms of plant population, we found a slight effect of the study site, possibly related to the plants' density. Matasgordas had a plant density 3 times higher than Martinazo, fact that could impinge the larvae presence and abundance. Jakobsson et al. (2009) stipulated that local flower density had an important mitigating effect on pollination success, as the probability of pollinator visits per flower decreased at higher flower densities. Thus, dwarf palms in high-density neighborhoods had lesser probability to hold weevil larvae due to a dilution effect, because weevils will visit and oviposit in few inflorescences. This negative relation with flower density has been described for the globe flower *Trollius europaeus* and its specialist pollinator and seed predator the fly *Chiastocheta* spp. (Klank et al., 2010).

In terms of spatial patterns, it could be expected for insects distribution to mirror the distribution of their food resources (Widenfalk et al., 2012a). In this study we were unable to detect any spatial pattern in the larvae occupancy patterns. Although plant selection by an insect (e.g. suitable oviposition sites) has been described as a major cause of nonrandom patterns in insect-host associations (Davis et al., 2004; Gascoigne et al., 2009; Groom, 1998; Kunin, 1993; Singer et al., 2002), and the dwarf palm follows a contagious pattern (Jácome-Flores et. al., unpublished), we found that this has no effect over the larvae occupancy patterns. This could be explained by the fact that the pollinator has a high mobility between plants. This was demonstrated with a "natural experiment" caused by a fire in 2012, when 97% of dwarf palms in Matasgordas study site were burned and most inflorescences with weevil larvae were eliminated. Fortunately, 95% of the burned palms survived, due to their capacity to resist fires (Granados et al., 1988; Herrera, 1989). The next year flowering occurs normally and adult weevils coming from unburned palms were capable to reach even the more distant breeding dwarf palm (235 m) in the study site, ovipositing in the same proportion as the previous year. This random pattern has been described by Elzinga et al. (2005) in the nursery pollination system *Silene latifolia* / *Hadena bricuris* where the parasitism rate was spatially uniform across the target population. Also, Widenfalk et al. (2012) showed no spatial pattern of gall (*Contarinia vincetoxici*) abundance over the host plant (*Vincetoxicum hirundinaria*).

In summary, our results demonstrate that in dioecious species with nursery pollination, plant sex, flowering display and flowering synchrony act as additive forces that influence the presence

and abundance of pollinator larvae. Furthermore, despite the fact that *D. chamaeropsis* use mainly male inflorescences, we found evidences that female dwarf palms also provide rewarding oviposition sites, "paying" thus the pollination services. This study opens questions about the nursery pollination in dioecious species. In particular, measurement of the relation between the number of fruits and the number of weevil larvae would complete the results of our work. On the other hand, the spatial arrangement of floral resources in highly synchronic populations seems not influence the larvae occupancy pattern, at least when pollinators have high mobility. Our findings of the occupancy patterns of the nursery pollinator contribute to understanding the mutualism between *C. humilis*/*D. chamaeropsis*.

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APPENDIX

Table 1. Type III effects. Relevance of the individual's inflorescences traits for the larvae abundance and presence in 2013. The models tested are form the proposed hypothesis, and the best models are in bold. Sex (S), number of inflorescences/individual (Ni), synchrony index (Sn) and study site (SS). ΔAICc is the relative difference of a given AICCc value compared with the smallest AICc. AIC weights indicate the relative support for every model (the weights of all the models in the candidate set have the sum of 1.

Models	AICc	ΔAICc	wAICc	Rank
Presence Models				
1) Sex	1117.56	18.04	0.0001	10
2) S + Ni + Sn	1109.47	9.95	0.0036	9
3) S + Ni + Sn + SS	1105.52	6	0.0257	6
4) S + Ni + Sn + SS + S*Ni	1104.28	4.76	0.0478	3
5) S + Ni + Sn + SS + S*Sn	1107.27	7.75	0.0107	8
6) S + Ni + Sn + SS + Ni*Sn	1104.42	4.9	0.0446	4
7) S + Ni + Sn + SS + S*Ni + S*Sn	1105.09	5.57	0.0319	5
8) S + Ni + Sn + SS + S*Ni + Ni*Sn	**1099.52**	**0**	**0.5166**	**1**
9) S + Ni + Sn + SS + S*Sn + Ni*Sn	1106.07	6.55	0.0195	7
10) S + Ni + Sn + SS + S*Ni + S*Sn +	1100.07	1	0.2995	2
Abundance Models				
1) Sex	2019.82	11.32	0.0022	9
2) S + Ni + Sn	2012.89	4.42	0.0700	6
3) S + Ni + Sn + SS	2011.12	2.59	0.1748	5
4) S + Ni + Sn + SS + S*Ni	2010.79	2.31	0.5520	4
5) S + Ni + Sn + SS + S*Sn	**2008.48**	**0**	**0.253**	**1**
6) S + Ni + Sn + SS + Ni*Sn	2012.98	4.5	0.033	7
7) S + Ni + Sn + SS + S*Ni + S*Sn	2008.77	0.29	0.267	2
8) S + Ni + Sn + SS + S*Ni + Ni*Sn	2014.35	5.87	0.016	8
9) S + Ni + Sn + SS + S*Sn + Ni*Sn	2010.42	1.94	0.117	3
10) S + Ni + Sn + **SS** + S*Ni + S*Sn +	2010.79	2.31	0.2010	4

Table 2. Type III effects. Relevance of the individual's inflorescences traits for the larvae abundance and presence in 2013. The explanatory variables for each response variable are those included in the best supported models.

		Presence	
Explanatory variables	**D. F.**	**F**	**P-value**
Sex	**1, 529**	**27.3**	**<0.0001**
Ninflor	**1, 529**	**4.23**	**0.0401**
Synchrony	1, 529	0.63	0.4268
Study site	**1, 529**	**4.51**	**0.0341**
Sex * Ninflor	**1, 529**	**6.37**	**0.0119**
Ninflor * Synchrony	**1, 529**	**6.17**	**0.0133**
		Abundance	
Sex	**1, 135**	**14.86**	**0.0002**
Ninflor	1, 135	1.19	0.2782
Synchrony	**1, 135**	**17.35**	**<0.0001**
Study site	**1, 135**	**4.23**	**0.0416**
Synchrony * Sex	**1, 135**	**4.65**	**0.0328**

CAPÍTULO 3

EFFECTS OF DENSITY, PLANT AGGREGATION AND SPATIAL GENETIC STRUCTURE ON POLLINATION SUCCESS OF A NURSERY-POLLINATED PLANT

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RESUMEN

El éxito de polinización suele estar relacionado con la segregación espacial, la densidad floral y estructura genética espacial de las poblaciones de plantas. En estudios previos se ha visto una variedad de efectos de estas variables que van desde efectos positivos a efectos neutros o negativos. Aunque, los sistemas de polinización obligada y especializada suelen ser más propensos a experimentar los efectos de la agregación espacial, la densidad de plantas y estructura genética espacial existen pocos estudios sobre ello. Analizamos observacional y experimentalmente las consecuencias de la distribución espacial, la densidad de población y la distribución de genotipos sobre el éxito de polinización (iniciación de frutos y frutos desarrollados) de dos poblaciones de *Chamaerops humilis*. Encontramos que la distribución espacial de las plantas no tuvo ningún efecto sobre el éxito de polinización. Este resultados puede ser explicado por el hecho de que los insectos polinizadores pueden viajar grandes distancias y no tienen preferencias por individuos florales agregados. Encontramos los genotipos de las plantas en ambas poblaciones tenían una distribución aleatoria indicando que no había ninguna limitación por calidad de polen. En los experimentos de polinización a mano, el origen del polen no tuvo ningún efecto sobre el éxito de la polinización; sin embargo, si encontramos que las plantas en altas densidades podrían experimentar competencia por nutrientes y polinizadores. Los resultados de este estudio sugieren que en un sistema de polinización especializado sin limitación de calidad de polen, el éxito de polinización dependerá de la cantidad de polinizadores especializados y de la depredación pre-dispersiva de semillas.

ABSTRACT

Pollination success is usually related to floral density, plants aggregation and spatial genetic structure (SGS) of plant population. In previous studies a variety of outcomes have been reported from positive to neutral or negative effects. However, these effects have been poorly studied in obligate specialized pollination systems. In this study, we analyze observational and experimentally the consequences of these variables over the pollination success (fruit initiation and fruit development) of two *Chamaerops humilis* populations. During two fruiting season we monitored the pollination success and performed hand pollination experiments. We found that the population with higher plant density had a lower fruit set and could be the result of an increased plant pollinator competition. However, an increase of intraspecific competition and differential allocation of resources for reproduction could be decreasing the fruit set. We found that plants in both populations had a random distribution of the genotypes (i.e. without a SGS), a relevant result that suggests there was no quality pollen limitation. This was corroborated with the results of hand-pollination experiments, as the origin of the pollen had no effect over pollination success. However, we found that plants experience a slight quantity pollen limitation. The results of this study suggest that when pollen quality was not a limitation, the aggregation level of the plants has no effect over the fruit set.

INTRODUCTION

Insect pollinated plants are especially sensitive to variations in population attributes concerning the spatial distribution of individuals. Pollination success is particularly affected by plants density (number of individuals per unit area) (Mustajärvi et al., 2001; Spigler and Chang, 2008), spatial distribution of individuals (aggregation level) (Condit et al., 2000; Fedriani et al., 2015; Wiegand et al., 2007) and spatial genetic structure (high genetic similarity among neighbors) (Hardy et al., 2006; Vekemans and Hardy, 2004). As a consequence, these variables play an important role in determining variation in reproductive success of plant populations (Davis et al., 2004; Groom, 1998; Kunin, 1993).

Plant populations with high density patches tend to enhance pollination success offering more resources to pollinators, increasing pollen deposition per flower and fruit initiation (Bosch and Waser, 2001; Cartar and Real, 1997; Kunin, 1997, 1993; Sih and Baltus, 1987). At plant level, several studies have shown that most insects move relatively short distances, thus, aggregated individuals are more often visited by effective pollinators and produce more fruits than less aggregated individuals (i.e. positive density dependence; Aizen and Harder, 2007; Fox, 2007; Nielsen and Ims, 2000). Nonetheless, this positive effect on reproduction in both high density and aggregated plants is not always in that way and could act different through different pollination stages (fruit development). There are various studies that shown a negative relationship between population density and spatial aggregation over pollination success (Carneiro et al., 2006; De Lacerda et al., 2008; Degen et al., 2004; Hardy et al., 2006). For instance, high-density populations can experience high pollinator services competition lowering fruit initiation and competition for resources with consequences on fruit development (Gunton and Kunin, 2009; Harper, 1977; Spigler and Chang, 2008).

In addition, the degree of genetic relatedness of pollen donors and receptors might have important consequences on fruit initiation and development. In plant aggregation, genetic similarity is often high leading to spatial genetic structure (hereafter SGS) with major consequences on plant reproductive success (Fedriani et al., 2015; Jump and Peñuelas, 2006). When populations present SGS, mating occurs among highly related neighbors, which results in

low quality of available pollen and lessened plant reproductive success by inbreeding depression (Ishihama et al., 2006). Thus, aggregated plants could experience higher fruit initiation, but low fruit development duet to a higher abortion rate (Fedriani et al., 2015). Whether these factors have an overall net positive or negative effect on individual fitness is an important question necessary to fully understand how plant populations are regulated.

Plants with highly specialized pollination systems should be most likely to experience the effects of plant density, spatial aggregation and SGS. However, there are few studies that assess these effects and its interaction (e.g. Després et al., 2007; Elzinga et al., 2005; Klank et al., 2010). One interesting system is the nursery pollination where plants provide egg-laying sites (e.g. seeds, ovaries, inflorescences) for the pollinator larvae development in exchange of pollination services (Anstett et al., 1997; Cook and Rasplus, 2003; Dufaÿ and Anstett, 2003; Herre et al., 2008). Host plants in dense patches should experience high competition for pollinator availability, as the pool of potential pollinators is assumed to be smaller than in the case of plants with generalized pollination systems (Johnson et al., 2012). Aggregated flowering plants may induce competition if local pollinators are saturated and their availability limits fruit set (Zimmerman, 1980). Although specialized pollinators provide high-quality outcross pollen (Schleuning et al., 2011) in populations with a highly SGS most mating occurs among highly related neighbors lowering the quality of available pollen (inbreeding depression) and lessened plant reproductive success (Aizen and Harder, 2007; Ishihama et al., 2006).

In this study we focused on the dioecious dwarf palm *Chamaerops humilis*, that is engaged in a nursery pollination system with the weevil *Derelomus chamaeropsis* (Anstett, 1999; Dufaÿ and Anstett, 2003; Dufay et al., 2004), apparently complemented by wind pollination (Dufaÿ and Anstett, 2004; Herrera, 1989). In a Mediterranean area of southern Spain, dwarf palms occur highly aggregated at different densities (Jácome-Flores et al., unpublished). We study two natural populations where we expect different effects of density, aggregation level and SGS over pollination success expressed at fruit initiation and fruit development. Distinguishing between fruit initiation and development facilitate the identification of different density-dependent mechanisms acting on individual pollination success (Fedriani et al., 2015). Our predictions were: (1) Plants in high densities can experience high competition for resources or pollinators (Gunton

and Kunin, 2009); thus, we expect that plants in the high-density study site will have lower fruit initiation. (2) Most insects move relatively short distances and tend to be more attracted to aggregated plants (Aizen and Harder, 2007) (Aizen & Vazquez 2006), thus, we expect that aggregated dwarf palms will have higher fruit initiation. (3) Dwarf palm spatial aggregation pattern and limited seed dispersal could result in a SGS (Vekemans and Hardy, 2004); as consequence, we expected that aggregated dwarf palms will experience pollen quality limitation, decreasing fruit development (e. g. Fedriani et al., 2015). We also performed hand-pollination experiments in aggregated and isolated plants for comparison. We expected that inflorescences hand-pollinated will have more fruit initiation that the open-pollinated, because the last one will experience pollen quantity limitation. Also, we expected that inflorescences pollinated with pollen of nearby plants would have more fruit abortion and lower fruit development (i.e. fruits that complete development) (Aizen and Harder, 2007; Herlihy and Eckert, 2004).

MATERIAL AND METHODS

Study species and area

Chamaerops humilis is a small (usually ~1.5 m high) dioecious palm (Herrera, 1989), that occurs only in the Mediterranean basin. In our study area, the Doñana Park, *C. humilis* is generally associated with sandy soils, presenting highly fragmented distributions due to both historic (crops, villages) and environmental (marshes, dune system) barriers. This species has a contagious and superposed spatial pattern, characterized by big clusters containing smaller ones and some individuals distributed randomly (Jácome-Flores et al., unpublished). Flowers are borne in dense inflorescences that develop within a single enlarged bract. It blooms during February-May and the number of flowers per inflorescence is highly variable for both male and female plants (9-810 flowers/inflorescence; unpublished data). At anthesis, both male and female plants attract pollinators with floral-like chemical compounds that curiously are released by leaves, and not by flowers (Dufaÿ et al., 2003). It has a mixed wind and nursery pollination system with the weevil *D. chamaeropsis* (Anstett, 1999; Herrera, 1989). In this nursery pollination system, once pollinating weevils have found a plant, they typically stay until the end of its anthesis, finding shelter, egg-laying sites and food within the inflorescences (Dufaÿ et al., 2004, Jácome-Flores et

al.,, unpublished). The fruits are polydrupes comprising one to three drupes. Nevertheless, we applied the term fruit to each drupe, as in fact it behaves as an independent pollination and dispersal unit. The initial fruit set happens between May-June, followed by the fruit development (June-August) and ending with fruit ripening (September-November).

The study was carried out at the Doñana National Park (510 km^2; 37º9' N, 6º26' W) located on the right bank of the Guadalquivir estuary in southwestern Spain. Average annual temperatures ranges between 15.37 and 18.67 ° C (mean = 16.91 ± 1.06 º C; n =35; period 1978-2013). Similarly, annual rainfall varied during this period between 170 and 1028 mm (mean=542.6 mm ± 12.02 mm; data from Monitoring Team of Natural Process of Doñana Biological Station; http://www-rbd.ebd.csic.es/Seguimiento/mediofisico.htm). We chose three study sites separated by 3-15 km within the Doñana area, called Matasgordas, Martinazo and El Puntal (Fig. 1). In these areas we selected and delimitated two observational and two experimental study sites, explained below.

Observational study sites. 1) Matasgordas study site was within a homogeneous area occupied by a dehesa. This dehesa (~ 300 ha) is a continuum of grasslands with open tree stratum of *Quercus suber* and *Olea europaea* var. *sylvestris* (Fedriani et al., 2010). In this site we delimited an observational study site of 22.1 ha with 294 individuals (~13 plants/ha). 2) Martinazo study site is dominated mainly by a pyrophitic scrub (*Halimium halimifolium*, *Rosmarinus officinalis*, *Ulex* spp. and *Stauracanthus* spp.) (Granados et al., 1988). Within this study site we delimitated an observational study site of 20.93 ha with 91 individuals (~4 plants/ha).

Experimental study sites. 1) In Matasgordas close to the observational study site, we delimited an experimental plot (~1.5 km^2) with 30 breeding females. 2) El Puntal is part of a marsh-xerophytic scrubland ecotone used to breed livestock. This area is characterized by meadows, fern covered ground and low shrubs (*Lavandula stoechas, H. halimifolium* and *C. humilis*) with sparse *Q. suber* trees (Cobo and Andreu, 1988). In this location we delimited an experimental study site (~2km^2) with 30 breeding females.

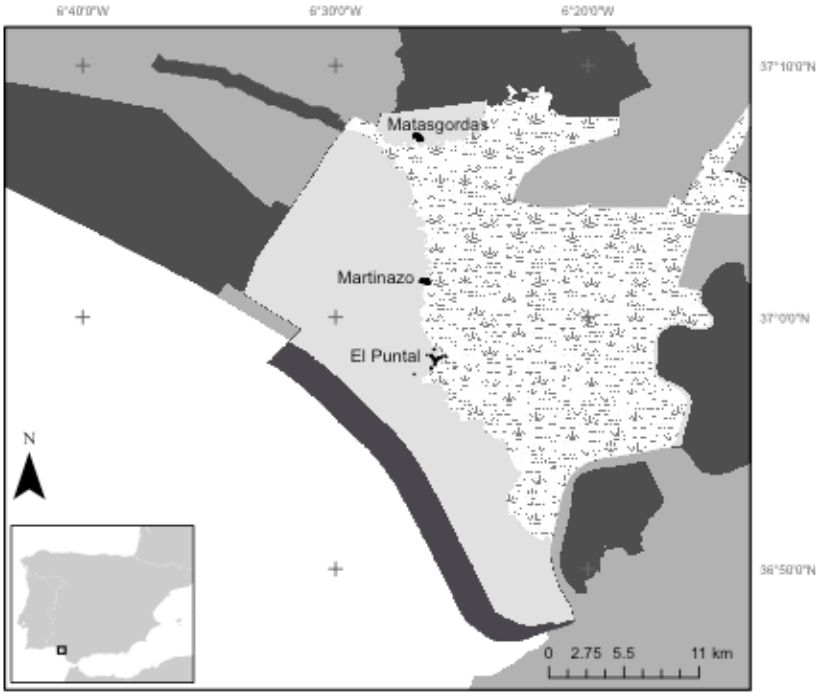

Figure 1. The three localities limited by the marshland (grass pattern) within the Doñana National Park area (lightest grey).

Pollination success observation

To estimate observationally the pollination success, during two flowering and fruiting seasons (February-August, 2012-2013), we monitored twice per month the inflorescences of all breeding females in the observational study sites (Matasgordas and Martinazo). The number of these females varied between years and study sites (Table 1). For both seasons, we selected randomly two-three inflorescences per plant. We estimated the pollination success via both fruit initiation (i.e. ovaries that set fruit / initial number of fruits) and fruit development (i.e. number of fruits that completed their development until late August/number of set fruits).

Table 1. Overall number of plants, inflorescences and drupes used in this study. The final column has the differences among the average drupes per inflorescence in each study site and year. Iso = isolated, Agg = aggregated.

Study site	Year	Females	Inflor.	Inflorescences (Iso./Agg.)	Drupes (Total)	Drupes (drupes/inf.)
Martinazo	2012	34	68	24 / 44	11325	111.89 ± 9.76
	2013	26	52	20 / 32	7584	150.88 ± 13.25
Matasgordas	2012	84	166	55 / 111	29141	142.77 ± 6.53
	2013	129	342	135 / 207	45561	114.47 ± 5.19

We used the results from the SPPA obtained from Jácome-Flores et al. (Unpublished) performed in the same areas to set a threshold of aggregation among isolated and aggregated females. According to these analyses, the average radius of clustering among individuals is 19-22 m. Thus, reproductive females with neighbors at distances below 22 m were considered as aggregated. On the other hand, to increase the isolation effect we selected as isolated females those without any conspecific in a radius of 30 m.

Dwarf palm spatial genetic structure

To examine the spatial genetic structure of the dwarf palm we collected leaf tissue of 100 randomly georreferenced individuals at Matasgordas and the 91 individuals at Martinazo. Leaf tissue was dried in silica gel. DNA was extracted of all the leaf samples and 12 nuclear microsatellite markers were tested for polymorphism (GF112060, GF112062, GF112063, GF112064, GF112070, GF112071, GF112072, GF112074, GF112076, GF112078, GF112082, GF112083; Arias et al., 2012). We performed polymerase chain reactions (PCR) in 25 µl of reaction mixture containing 10 ng of template DNA, 1 × PCR buffer, 2 mM MgCl 2, 0.25 µM fluorescently labeled (6-FAM, VIC, NED, and PET dyes) forward primer, 0.25 µM reverse primer, 0.2 mM dNTP and 0.75U/µl Taq polymerase. The PCRs were performed in a Biometra Gradient Thermal Cycler, with an initial 2 min of denaturation at 94° C, 35 cycles at 94° C for 1min,

annealing at different temperatures depending on the marker (52°C for GF112060 and GF112070; 55°C for GF112062, GF112063, GF112064, GF112071, GF112072, GF112074, GF112076, GF112082 and GF112083; 57°C for GF112078) for 1 min, and extension at 72 ° C for 2 min; and a final extension at 72 ° C for 10 min. Polymerase chain reaction products were analyzed on an ABI 3130X1 Genetic Analyzer and sized using GeneMapper v.4.0 (Applied Biosystems, Foster City, California, USA) and GeneScan 500 LIZ size standard. We genotyped 17 individuals twice for each locus to calculate the mean genotyping error rate per locus that was 1.0%.

Hand pollination experiments

To estimate experimentally the pollination success, during two flowering and fruiting seasons (February-August 2012-2013), we selected a sample of 15 aggregated breeding females and 15 isolated in each experimental study site (El Puntal and Matasgordas). For the different pollination treatments we randomly selected four inflorescences in aggregated plants and three in isolated (see below). To prevent natural fertilization by any pollinator (*D. chamaeropsis*), before any pollination experiment mesh bags were placed in prophylls before their opening, securing with a mooring and silicone grease (e.g. Hansen et al., 2007) as an adhesive on the basis. The mesh size used in the bags was 0.061 mm, big enough to let it pass the dwarf palm pollen grains with mean: 19.75 S.E. ± 1.47 μm potentially transported by the wind. Once the inflorescences enter in anthesis, hand pollination was performed with the help of a cotton-tipped swab, which was different for each inflorescence (e.g. Ortiz et al., 2006). The treatments were: 1) wind pollinated, inflorescence covered for the full flowering period to determine the contribution of the wind-pollination; 2) inflorescences pollinated with pollen from two-three males belonging to the group (only for aggregated); 3) inflorescences pollinated with pollen from a pool of distant males; 4) natural pollinated, inflorescence uncovered during all flowering season.

Notice, that hand pollinated inflorescences were covered and secured again after the pollen addition to exclude any external pollen source. Also, as pollen from remote males we used a mix of pollen of one inflorescence of two-three staminate males, located at distances between 100-500 m far away from all females. This minimizes the possible individual effects, due to the different

qualities of pollen. From each staminate inflorescence we collected the pollen in a plastic container.

Statistical analysis

The data of both fruit initiation and fruit development were analyzed by fitting generalized linear mixed models (GLIMMIX; Littell et al., 2006). Residuals of the response variable (e.g., fruit set) were assumed to follow a binomial distribution and thus and we used logit link function. This procedure allows the modeling of non-normal response variables, as well as the use of both fixed and random factors. Individual plant, inflorescence nested within plant and year were included as random factors. For the observational data we analyzed the effect of the aggregation level and study site on the fruit set. In the case of pollination experiments, we analyzed the effect of the pollination treatment in aggregated and isolated females. We included the pollination treatment (remote, nearby and natural pollination), spatial distribution of individuals (aggregated vs. isolated), study site and the second order interactions as fix factors. The interaction between pollination treatment and spatial distribution indicates if supplementing pollen from a remote donor benefited more to aggregated than to isolated individuals. We calculated the adjusted means and standard errors using the LSMEANS statement and were back-transformed using the appropriate Taylor's series approach (Littell et al., 2006). When one factor was statistically significant, a posteriori contrast analysis was done to detect the main differences between factor levels

In relation to SGS, for each study site we estimated the mean number of alleles per locus (n_a), using the program GENALEX v.6 (Peakall and Smouse, 2006). We explored the genetic differentiation by means of nested analyses of molecular variance (AMOVA; Excoffier et al., 1992), among populations within areas (F_{IS}), among individuals within populations (F_R) and within individuals. All F – statistics (Weir and Cockerham, 1984; Wright, 1950) and their significance, based on 999 permutations of individuals, were estimated with GENALEX. Finally, we estimated the genetic differentiation, with a partial Mantel test controlling for the log-transformed geographical position of populations and individuals given.

RESULTS

Pollination success observation

Fruit initiation was low at both observational study sites, although 1.42 times higher in Martinazo (0.17 ± 0.022) than in Matasgordas (mean ± SE; 0.12 ± 0.038) (Fig. 2), suggesting some effect of density. We found significant differences ($F_{1, 422}$=3.91, P=0.0486) between the two study sites, but not any relevant effect neither of plant aggregation level ($F_{1, 422}$=1.21, P=0.2718) nor of its interaction with the study site ($F_{1, 422}$=0.29, P=0.5891).

Fruit development was high in both study sites, with significant differences ($F_{1, 89}$=16.3, P=0.0001) between them. In Matasgordas fruit development set was 0.677 ± 0.0.095, and in Martinazo 0.8089 ± 0.068 (Fig. 2). Also, there were no relevant effects of the aggregation ($F_{1, 89}$=3.86, P=0.0526) neither of the interaction between aggregation and site ($F_{1, 89}$=2.08, P=0.1526).

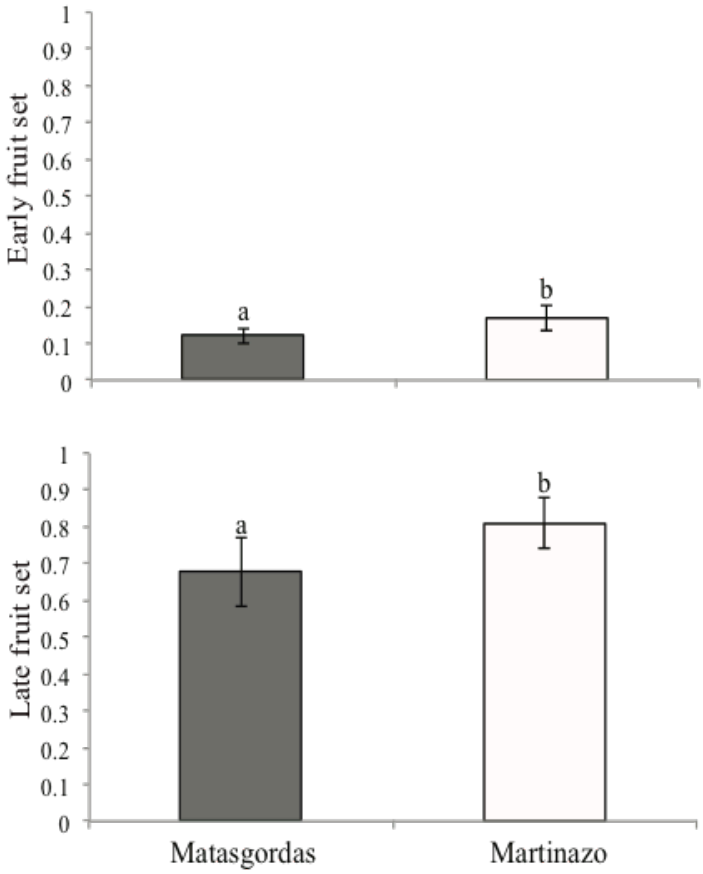

Figure 2. Results of early and late fruit set proportion in both observational study sites based in fruit set proportion. Error bars represent standard errors. Different super index over the error bars indicate significant differences (p<0.05).

Spatial genetic structure

The mean number of alleles per locus was 6.1 ± 1.2. Inbreeding coefficients (F_{IS}) were positive in both study sites (Matasgordas= 0.114 and Martinazo= 0.219). The AMOVAs show that most of the genetic variation occurred within individuals in both populations (89% in Matasgordas and 78% in Martinazo) and when analyzed together (82%), with 0% of variation among populations. This lack of genetic differentiation among populations was confirmed by the non-significant FST (FSR = 0.001, P=0.234). Only 3% of the individuals were clones. The Mantel test results show no significant spatial associations among geographic individuals distance and its genetic differences in Matasgordas (P=0.417). Instead, in Martinazo we found a slight but still no significant effect (P=0.07). Thus, an effect on pollen quality of the spatial genetic structure, through inbreeding depression, should not be expected.

Hand-pollination experiments

The percentage of inflorescences covered with mesh bags that successfully gave rise to fruits was very low (<0.09 %), suggesting wind pollen contribution was irrelevant. The data corresponding to these inflorescences were excluded from later analyses.

The pollination treatment had a relevant effect on fruit initiation ($F_{1, 79}$= 152.53, P<0.001), being the hand-pollinated fruit set higher (0.22) than the natural pollinated one (0.16) (Fig. 3A). The other relevant effect was the second-order interaction of pollination treatment and plant aggregation level ($F_{1, 79}$= 21.91, P<0.001). Differences on fruit initiation between hand and natural pollinated inflorescences were higher for aggregated individuals (test of slices $F_{1, 79}$= 163.6, P<0.001).

Also, the fruit set was low (<0.24) in the experiments using pollen from nearby and remote males, without significant effect of the treatment ($F_{2, 103}$= 1.88, P=0.1573) (Fig. 3B).

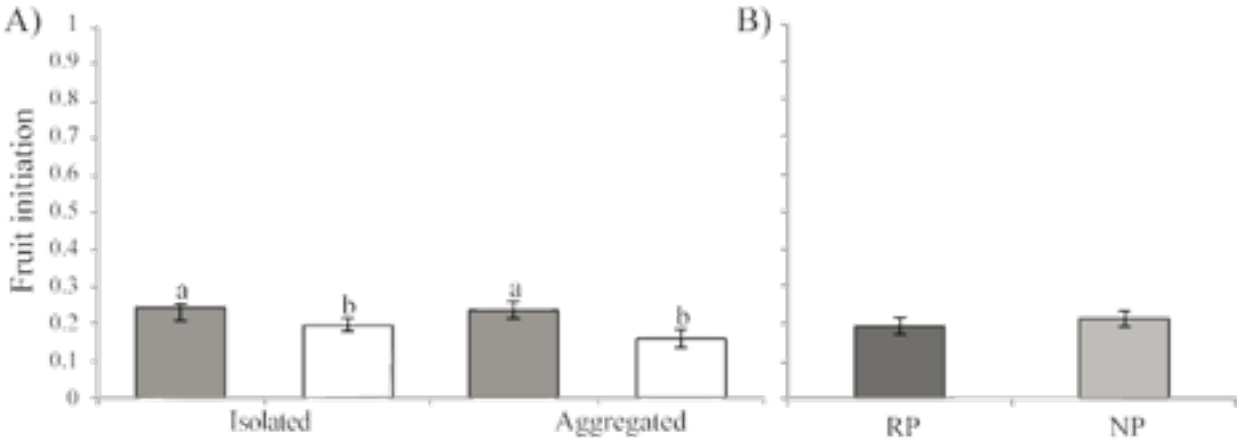

Figure 3. A) Results of fruit initiation in isolated and aggregated individuals based on the pollination treatment. Grey bars correspond to hand-pollinated ones and white bars to natural pollinated. B) Average fruit initiation proportion in aggregated females based on the pollen treatment. Remote pollen (RP) and Nearby Pollen (NP). Error bars represent standard errors. Different super index over the error bars indicate significant differences ($p<0.05$).

In the fruit development, we also found that the pollination treatment had a significant effect ($F_{1, 80}$=31.73, P<0.001), however, natural pollinated plants had higher values (0.57) than hand-pollinated ones (0.49) (Fig. 4A). Also, there was a significant effect of the interaction between pollination treatment and aggregation level ($F_{1, 80}$=4.16, P=0.0448). The differences on the pollination treatment were more marked in aggregated (test of slices $F_{1, 80}$= 33.17, P<0.001) individuals.

We found no significant effects of the pollination treatment (from nearby or remote males) on the fruit development of aggregated palms ($F_{1, 15}$= 0.25, P=0.2096).

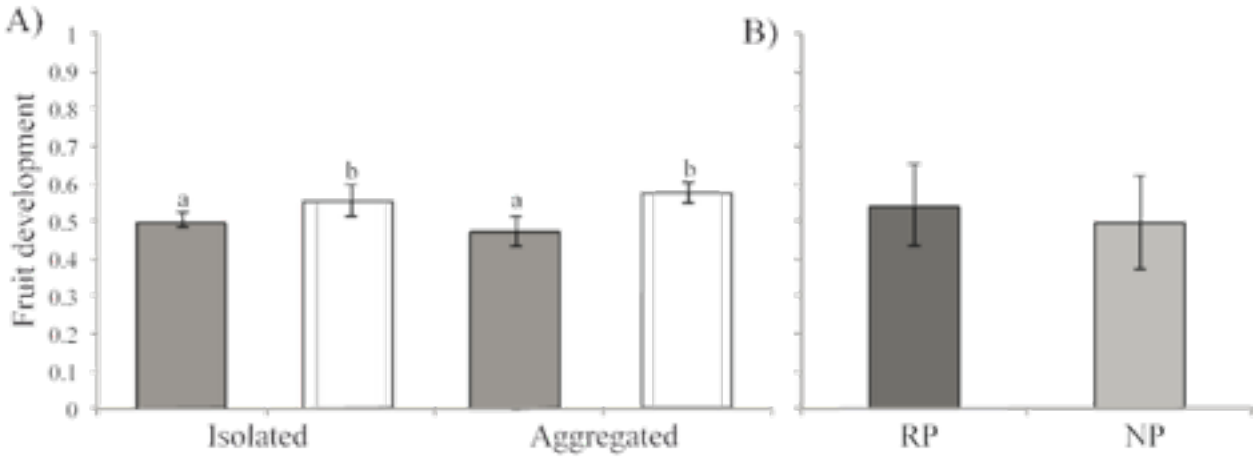

Figure 4. A) Results of fruit initiation in isolated and aggregated individuals based on the pollination treatment. Grey bars correspond to hand-pollinated ones and white bars to natural pollinated. B) Average fruit initiation proportion in aggregated females based on the pollen treatment. Remote pollen (RP) and Nearby Pollen (NP). Error bars represent standard errors. Different super index over the error bars indicate significant differences ($p<0.05$).

DISCUSSION

In particular, we found that the relationship between density and pollination success was negative: low-density population in Martinazo had more fruits. Negative effects of intraspecific interactions via competition in large populations of plants are well documented in the literature (Craine, 2005; Harper, 1977; Mustajärvi et al., 2001; Padien and Lajtha, 1992; Tilman, 1994; Vilà and Sardans, 1999). Dwarf palms in high-density populations could be experiencing the direct effect of competition for resources such as light, water and nutrients these resources (Gunton and Kunin, 2009; Metcalfe and Kunin, 2005). This result might arise due to dwarf palms in Martinazo offer in general more ovaries per inflorescence than Matasgordas (Table 1). Mustajärvi et al., (2001) and Metcalfe and Kunin (2005) found that low-density populations had more flowers per inflorescence and more ovaries per flower compared to dense populations. This pattern was caused by an increase of resources availability in plants in low-density (Stanton, 1987) and an active resource allocation to reproductive structures to attract pollinators. Density had other important mitigating effect on pollination success, as the probability of pollinator visits per flower decreased at higher densities lowering fruit initiation (Delmas et al., 2014; Fritz and Nilsson, 1994; Jakobsson et al., 2009; Larson and Barrett, 2000). However, through our hand-pollination experiments we found that the effect of pollen quantity limitation is very small. Thus, resource

competition and resource allocation could be the two factors that explain better the pollination success.

The spatial aggregation of the plants influences the pollination success positively (Aizen and Harder, 2007; Nielsen and Ims, 2000) and negatively (Fedriani et al., 2015). However, in our *C. humilis* populations we were unable to found any effect of the spatial aggregation of the plants. This could be related to the travel distances that the weevil transport pollen and the lack of (SGS). Weevil pollinators can travel long distances (Jácome-Flores et al., unpublished) with apparently no preferences for aggregated or isolated floral individuals. Furthermore, in ongoing spatial patterns analysis we found that pollination success had high positive spatial autocorrelation at distances up 40 m. Thus, the aggregation radius used to detect an effect of the spatial distribution was insufficient.

Contrary to our prediction, our genetic analysis indicates that, in spite of the strong aggregated pattern in both study sites, less 3 % of the individuals were clones. Furthermore, spatial autocorrelation analysis showed that both study sites have a random SGS. In Martinazo show slight but still no significant effect between spatial associations among geographic individuals distance and its genetic differences. Plants density is expected to play a major role in SGS because low densities, should result in increased SGS because of higher local genetic drift (Jump and Peñuelas, 2006; Vekemans and Hardy, 2004). However, pollen dispersal distance can offset the effect of genetic drift and modify SGS (Ennos, 1994; Escudero et al., 2003). Thus, an increase in gene dispersal distances, could reduce SGS resulting in a random or near random distribution of the genotypes (Born et al., 2008; Dick et al., 2003; White et al., 2002). Thus, in our study system it appear that two mechanisms increase the gene flow and random SGS: 1) pollinators weevil have high mobility and can travel and transport pollen more than 200 m (Jácome-Flores et al., unpublished) and 2) the long-distance dispersal by badgers (*Meles meles*) and red foxes (*Vulpes vulpes*) can move the seeds various kilometers (Fedriani and Delibes, 2011; Fedriani et al., 1999; Revilla and Palomares, 2002). The random SGS means that our studied populations did not experience pollen quality limitation through inbreeding depression. Thus, explaining why we found high fruit development, where more than 70% of the fruits develop no matter the plant aggregation level. This result is surprising and contrary to some other similar self

incompatible plants with entomophilous pollination and highly aggregated spatial pattern, where pollen deposition is likely to come from genetically related conspecifics which can strongly contribute to qualitative pollen limitation, enhancing fruit abortion and abscission (Aizen and Harder, 2007; Fedriani et al., 2015, 2010; Harder and Barrett, 1996; Mezquida and Olano, 2013).

One important finding that we made through our exclusion and pollinated experiments is that we were able to discard wind as major pollination vector. Although, the dwarf palm pollination system has been described first as a wind-pollination system (Herrera, 1989) and then as an ambophilous by wind and insects (Anstett, 1999) there were not experimental approaches that corroborate it. Our results from the inflorescences bagging exclusion, demonstrate that pollen transported by the wind, pollinates successfully less than 0.1 % of the ovaries in the covered inflorescences. With this result we can state that the insect pollination performed. On the other hand, as we showed above in the experimental plots we found that fruit initiation had only 1.38 fold increase when inflorescences received manually a large load of pollen, suggesting that, if it exists, pollen quantity had a small effect (Aizen and Harder, 2007; Fleming et al., 2001). Usually, the studies that observe pollen quantity limitation report that hand-pollinated plants led to a more than two or three-fold increase in fruit production flower compared to natural-pollination (e. g. Becker et al., 2011). An interesting result in both observational and experimental sites is that the fruit initiation was less than 30 %. Each drupe has the capacity to form a single fruit, but, it appears that usually only one in three drupes in each flower is capable of developing. There are two mechanisms that can explain this pattern: 1) selective abortion with the objective of enhancing offspring quality by selection of superior genotypes (Kozlowski and Stearns, 1989; Stephenson, 1981); and 2) competition among developing zygotes (sibling rivalry) for maternal resources during embryo and endosperm development (Mock and Parker, 1997). Of course, there is the need to formulate experiments that test the causes of the differences between developing zygotes in each flower, as well as the probable physical constraints during fruit development (Obeso, 2004).

It was surprising and contrary to the observational results that inflorescences exposed to natural pollination showed higher fruit development. It is likely that the manipulation of these inflorescences had cause damage to fruits, leading to a fewer fruit development. Also, these inflorescences were covered and secured again after the pollen addition, this exclude any external

pollen source. Instead in the natural pollinated were open to repeated pollinator visits provided ample pollen that the single manual pollination event (Ashman et al., 2004; Petit, 2011).

In conclusion, it seems that when the pollen quality was not a limitation the aggregation level of the plants has no effect over the fruit set. Instead in highly dense dwaf palm populations the resource allocation and competition for the specialized pollinators could decrease pollination success. On the other hand, the low levels of fruit intiation in observational and experimental study sites could be the result of competition between siblings but there is the need to formulate experiments that test the causes of the differences between developing zygotes in each flower, as well as the probable physical constraints during fruit development (Obeso, 2004).

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CAPÍTULO 4:

INEQUALITIES IN VISIT FREQUENCY AND FRUIT HARVESTING OF A MEDITERRANEAN PALM BY FUNCTIONALLY DIFFERENT FRUGIVOROUS MAMMALS

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RESUMEN

La dispersión de semillas endozoócora, consiste en la producción de frutos carnosos para atraer a vertebrados que dispersan las semillas internamente lejos de la planta madre. La existencia de la pulpa como recompensa atrae no sólo a dispersores de semillas, sino también a un conjunto de distintos frugívoros que consumen los frutos sin dispersar las semillas o destruyéndolas. La correcta identificación de las interacciones planta-frugívoro es esencial para conocer la contribución de cada frugívoro en la dispersión de semillas sembrar-dispersión y el mantenimiento de las poblaciones de plantas. La distribución espacial de las plantas y sus frutos influyen en los patrones de frugivoría alterando la frecuencia de visitas de los frugívoros y la las tasa de consumo. En este estudio, se analiza cómo la distribución espacial del palmito *Chamaerops humilis* afecta a la frecuencia de visitas de sus frugívoros visitas y las tasas de remoción de frutos en dos tierras áreas dentro del Parque nacional de Doñana. Encontramos que la agregación espacial de plantas con fruto no tuvo ningún efecto sobre las tasas de visitas de frecuencia y las tasas de consumo de frutos. Sin embargo, encontramos diferencias entre los frugívoros los cuales responden de manera diferente a la agregación de las plantas. Encontramos que los cérvidos (depredadores de semillas) y conejos (despulpadores de semillas) tuvieron los más altos valores de la eficacia de consumo de frutos, lo que limita la dispersión de semillas. Por otro lado, los tejones consumieron proporcionalmente más frutos por planta que otros frugívoros en la asamblea, pero en general tuvo una eficacia media de consumo de frutos. Sin embargo, los frutos consume son esenciales la dispersión a larga distancia del palmito, facilitando la conectividad entre parches y el flujo de genes entre las poblaciones, la colonización de nuevas áreas y la expansión de la población. La consecuencia más evidente de los patrones frugivoría encontrados es que coinciden con los patrones distribución espacial del palmito donde los tejones crean grandes agregaciones de plantas, los conejos y roedores crean pequeñas agregaciones de plantas y los cérvidos contribuyen a la dispersión aleatoria de las plantas.

ABSTRACT

Endozoochorous seed dispersal relies on fleshy fruits to attract vertebrates that transport the seeds internally away from the mother plant. Fruit flesh attracts not only seed dispersers but also distinct frugivores that harvest fruits without dispersing the seeds or by destroying them. The correct identification of plant-frugivore interactions is relevant to know the contributions of every frugivore to seed-dispersal and the maintenance of the plant populations. Fruit resources spatial distribution can influence patterns of frugivory by affecting the rates of frugivore visits and fruit harvesting. In this study we analyze how the dwarf palm *C. humilis* spatial distribution affects the frequency of frugivorous species visits and the fruit-harvesting rate in two abandoned and pastoral lands within a world Biosphere Reserve. We found that spatial aggregation of fruiting plants had no effect on rates of visits and fruit harvesting. However, we found differences between frugivores. Cervids (seed predator) and rabbits (pulp feeders) had the highest values of fruit harvesting effectiveness, limiting efficient seed dispersal. Badger proportionally handled more seeds per visited plant than other frugivores in the assemblage, but in general handled few fruits. However, the few fruits handled by this species were essential for the dwarf palms long-distance dispersal facilitating inter-patch connectivity and gene flow among populations, slow colonization of new areas and population expansion. Spatial patterns of frugivory coincide with the spatial distribution patterns of the dwarf palm: badgers seem create large plant aggregations, rabbits and rodents small plant aggregations, and cervids contribute to the random dispersal.

INTRODUCTION

Seed dispersal represents the end of the plant reproductive cycle, with the subsequent establishment and recruitment of a new generation of plants in a more or less distant area (Nathan and Muller-Landau 2000, Wang and Smith 2002, Terborgh et al. 2008). This process provides the means for most plants to escape of increased seedling mortality under conspecific plants (Janzen 1970, Connell 1971, Howe and Smallwood 1982, Schupp 1988, Hammond DS 1998), reduces the effects of inbreeding depression (Vekemans and Hardy 2004, Bizoux et al. 2009) and allows the colonization of new habitats (Hewitt and Kellman 2002, Brunet 2007). In this context, seed dispersal influences the spatial distribution, regeneration, demography, and genetic structure of plants (Howe and Smallwood 1982, Nathan and Muller-Landau 2000, Herrera 2002, Dennis and Westcott 2006, Fedriani and Wiegand 2014).

A wide diversity of dispersal agents (both biotic and abiotic) can contribute to seed dispersal. One of the most extended mode of seed dispersal is endozoochory, which relies on fleshy fruits to attract vertebrates able to move the seeds internally away from the mother plant (Howe and Smallwood 1982, Kissling et al. 2009, Fedriani and Wiegand 2014). The existence of rewarding flesh attracts not only seed dispersers but also an assemblage of distinct frugivores that harvest fruits without dispersing the seeds or by destroying them (Howe and Smallwood 1982, Fedriani et al. 2012, Fedriani and Delibes 2013). In general, we can distinguish three type of functional frugivores in relation to the seeds: 1) legitimate dispersers, which perform successful seed dispersal; 2) ineffective or poor dispersers (pulp feeders), which leave defleshed seeds nearby o beneath the mother; and 3) seed predators, which grind and destroy the seeds. The rates of fruit harvesting by each group are variable and depend on their frequencies of visit to fruiting plants and the number of seeds handled (Vázquez et al. 2005, Burns 2006). Thus, the total number of seeds handled depends not only on interaction frequency, but also on the number of seeds handled per interaction, thus the fruit harvested effectiveness will be the product of these interactions (Schupp et al. 2010). There are many outcomes of this interaction, for instance, some visitors could harvest large amount of fruits per interaction but make infrequent visits, while highly frequent visitors could harvest scarce fruits, all of them having similar effectiveness (e. g. Fedriani et al. 2012, Sasal and Morales 2013). The correct identification of these interactions is relevant to

know the contributions of every frugivore to seed-dispersal and the maintenance of the plant populations.

Spatial distribution of plants and their fruit resources can influence patterns of frugivory by altering visit frequency and fruit harvesting (Levey et al. 1984, Sargent 1990, Saracco et al. 2005). However, there is no a general pattern; fruiting plants aggregation level could increase fruit harvesting by attracting more frugivores (Sargent 1990, Takahashi and Kamitani 2004), while in other cases it decreases fruit harvesting as plants compete for frugivores (Borgmann et al. 2004, Carlo and Morales 2008, Blendinger et al. 2008). Instead, isolated plants may be less attractive to frugivores, thus decreasing visit frequency and fruit harvesting rates (Takahashi and Kamitani 2004, Pizo and Almeida-Neto 2009), but also it could reduce the dilution effect increasing fruit harvesting rates (Moore and Willson 1982, Denslow 1987). Furthermore, the harvesting patterns of a given functional type could influence over those of other frugivores; for instances, pulp feeders could prefer feeding on aggregated plants (Moreno et al. 1996, Fedriani and Manzaneda 2005) lessening the fruist availability for seed–dispersers (Fedriani and Delibes 2013).

In this study, we analyze how plant spatial distribution affects the rates of frugivore visit and fruit-harvesting in two abandoned and pastoral lands within a world Biosphere Reserve. We selected the dwarf palm *Chamaerops humilis* and its frugivorous mammals in a Mediterranean scrubland (Doñana National Park; see below). This plant is an excellent model given its aggregated spatial distribution and capacity to colonize new habitats (Jácome-Flores et al., unpublished), as well as the functionally diverse assemblage of frugivorous mammals that harvested its fruits in Southern Spain (Fedriani and Delibes 2011, 2013). The dwarf palm fruits are usually harvested by a variety of frugivores, including legitimate seed dispersers (mainly carnivores), pulp feeders (rabbits and rodents) and seed predators (ungulates) (Fedriani and Delibes 2011). Detailed documentation of the services provided by frugivores is limited for uncommon animal vectors, such as carnivorous mammals (e.g., Herrera 2002, Fedriani and Delibes 2009), limiting our understanding of the level of generalization of many plant populations with respect to dispersers. We formulate various hypothesis related to the influence of the plants aggregation level on harvesting rates by the different functional frugivores. 1) Because aggregated plants tend to attract more frugivores (Sargent 1990, Takahashi and Kamitani 2004), we expected

that aggregated *C. humilis* would have higher frequency of frugivore visit,. 2) We expect that legitimate dispersers (badgers and foxes; Fedriani & Delibes 2011) would remove more fruits on isolated plants, because this plants would be less visited by other frugivores and thus would offer more available fruits (Moore and Willson 1982, Denslow 1987). 3) We predict that isolated plants would have less fruit harvesting by pulp feeders and seed predators, because they would prefer to feed on aggregated plants (Moreno et al. 1996, Fedriani and Manzaneda 2005). 4) We predict that seed predators and pulp feeders would have the highest fruit harvested effectiveness, because in general legitimate seed dispersers (carnivores) are less abundant and more infrequent visitors (Fedriani and Delibes 2009). Finally, we discuss the consequences of our results for the dwarf palm successful dispersal.

MATERIAL AND METHODS

Study species and area

Chamaerops humilis is a small (usually ~1.5 m high) dioecious palm (Herrera 1989), considered a thermo-Mediterranean bioindicator usually not present beyond 1000 meters above sea level, being most common in coastal areas. It is relatively abundant in Mediterranean scrub thickets and open pine forests. The dwarf palms have a strongly contagious pattern, characterized by a superposition patterns (big clusters, containing small ones) and some individuals randomly distributed (Jácome-Flores et al. unpublished). It blooms during March-May showing a nursery pollination system with the weevil *D. chamaeropsis* (Jácome-Flores et al., unpublished). Flowers borne in dense inflorescences developped within a single enlarged bract (Dufaÿ and Anstett, 2003). The fruits are "polydrupes" comprising one to three drupes that ripen in autumn (September–November). Nevertheless, we will apply the term fruit to each drupe, which in fact behaves as an independent dispersal unit (Fedriani and Delibes 2011). Fruits are attached to infructescences (or ramets) of up to 30 cm long (1–142 fruits per ramet, mean= 25.70 ± 1.92; unpublished data) and located at 10–30 cm from the ground level. Fedriani and Delibes (2011) described the main frugivores that consume dwarf palm fruits. Seeds were found in the feces of carnivores such as the Eurasian badger (*Meles meles*) and the red fox (*Vulpes vulpes*), which act as legitimate seed dispersers. On the other hand, European rabbit (*Oryctolagus cuniculus*) and some

rodents (*Mus spretus*, *Apodemus sylvaticus*, *Rattus rattus*, *Eliomys quercinus*) feed on the pulp of ripe fruits, generally leaving the defleshed seeds under or near the mother palms (Fedriani and Delibes 2011). Finally, ungulates like wild boars (*Sus scrofa*) and cervids (*Cervus elaphus, Dama dama*) generally depredated the seed of the ripen fruits, acting as seed predators.

The study was carried out during two fruiting seasons (2012-2013) at two *C. humilis* populations in the Doñana National Park (510 km^2; 37º9' N, 6º26' W), located on the right bank of the Guadalquivir estuary in southwestern Spain. We selected and delimitated two observational plots separated by 15 km within Doñana area, called Matasgordas and Martinazo (Fig. 1). Both areas have been affected by intense human activities, such as defaunation, land use change and periodical burnings (Granados et al. 1988). Matasgordas plot is within a dehesa generated in 1970 when all shrubs and most trees were mechanically removed (Figure 1). This management resulted in a continuum of grasslands with open tree stratum of *Quercus suber*, *Olea europaea* var. *sylvestris* and *Fraximus angustifolia* with no, or only sparse, understory of Mediterranean scrubs (Fedriani et al. 2010). The area was used for intensive cow grazing until 1996, when the land became public and the cows were removed, under the protection of the Spanish National Park Service. Since then, several mammal-dispersed plants, including *C. humilis*, are recolonizing the area. In this site we followed 48 females with ripe fruits. Instead, Martinazo has been used for livestock ranching, which increased the herbivory pressure over the native shrub. Additionally woody species (*Q. suber*, *O. europaea* var. *sylvestris*) were cut down, and controlled rotating burnings were applied every 25-30 years (Granados et al. 1986, 1988). Since then, the area has been recolonized by vegetation composed mainly by a pyrophitic scrub (*Ulex* spp., *Stauracanthus* spp. and *C. humilis*) (Granados et al. 1988). Within this plot we followed the ripe fruits of 24 females (Figure 1).

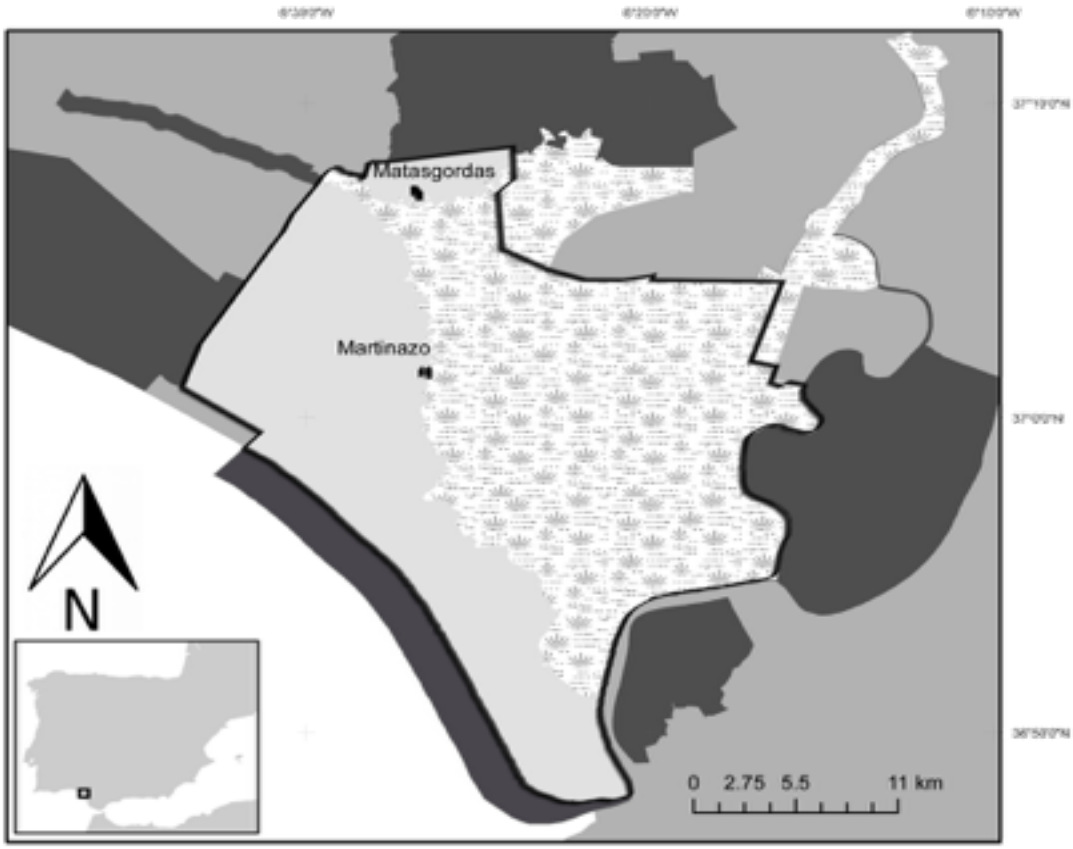

Figure 1. The two plots limited by the marshland (grass pattern) within the Doñana National Park area (lightest grey).

Frequency of visitors and fruit harvesting

Visit's frequency and fruit harvesting rates were estimated during the fruits ripening on two dispersal seasons (September-November) of 2012-2013. For each of the fruiting-female dwarf palms (see study area) we selected and followed the fruit-harvesting rate of two infructescences (in all, 1672 fruits). Fruits were counted and monitored every morning during twelve consecutive days. During this monitoring period we registered frugivores visits frequency through identification of their footprints in a semi-circular plot of sandy substrate (1 m diameter) surrounding the infructescences of each female (Mendoza and Dirzo 2007, Fedriani and Delibes 2009, 2013). The footprints were cleaned at the start of each twelve-day field experiment and again after each observation. When more than one species had visited a dwarf palm the identity of

the fruit remover was questionable. Therefore, we considered only those visits made by a single visitor, whose identification was conclusive (Fedriani and Delibes 2013).

To assess the effects of the aggregation level over the rates of fruit predation and dispersal we used the results from the SPPA obtained from Jácome-Flores et al. (Unpublished) performed in the same areas, to set a threshold of aggregation among isolated and aggregated females. According to these analyses, the average radius of clustering among individuals is 19-22 m. Thus, reproductive females with neighbors at distances below 22 m were considered as aggregated. On the other hand, to increase the isolation effect we considered isolated female those without any palm in a radius of 30 m. In Matasgordas we followed 9 isolated and 39 aggregated females, and in Martinazo 12 isolated and 12 aggregated females.

Statistical analysis

Differences in the visit frequencies to fruiting dwarf palms were evaluated by Fisher exact tests using SAS PROC FREQ (SAS Institute, 2003). We analyzed the proportion of harvested fruits by fitting a generalized linear mixed model using the Proc Glimmix from SAS (GLIMMIX; Littell et al., 2006). Residuals of the response variable (e.g., proportion of harvested fruits) will be assumed to follow a binomial distribution and thus and we will used logit link function. This procedure allows the modeling of non-normal response variables, as well as the usage of both fixed and random factors. We used as fixed factors frugivore species (badger, rabbit, rodents, cervids and wild boar), plants aggregation level and its second order interaction, considering only dwarf palms visited by a single frugivore during a particular night. Season (2012-2013), day (nested within season), plot (Martinazo and Matasgordas), individual plant (nested within plot) and infructescence (nested within plant and plot) were included as random factors. We calculated the adjusted means and standard errors using the LSMEANS statement and were back-transformed using the appropriate Taylor's series approach (Littell et al. 2006). When one factor was statistically significant, a posteriori contrast analysis was done to detect the main differences between factor levels. In the case of significant interactions, we tested for the effect of a given

factor at the different levels of the other factor (i.e. tests of slices). We performed using the SLICE option in the LSMEANS statement of the MIXED procedure (Littell et al. 2006).

RESULTS

Frugivore visitors

We found frugivore tracks in the 72 dwarf palms, where we recorded 425 visits generally performed by a single frugivore species (only 4.94% by two frugivores). From our mixed model, the main factor aggregation of plants ($F_{4, 1835}$= 2.38, P=0.0971) had no effect over the frugivore visit rate. However, we found differences in the visits frequency probability ($F_{4, 1835}$= 62.07, P<0.0001). Specifically, cervids had the highest visit (mean= 0.50 ± S. E. = 0.026), followed by rabbit (0.29 ± 0.024), rodents (0.14 ± 0.018), wild boar (0.08 ± 0.014) and badger with the lowest probability (0.05 ± 0.012) (Figure 2). The interaction between frugivore and aggregation had a significant effect ($F_{4, 1835}$= 2.38, P=0.0499). All frugivores visits frequency was different in isolated plants (test of slices, $F_{4, 1835}$= 33.08, P<0.0001) and aggregated plants (test of slices, $F_{4, 1835}$= 31.99, P<0.0001). Cervids had the highest visits frequency in both isolated and aggregated plants, followed by rabbits, rodents, wild boar and badgers (Figure 2).

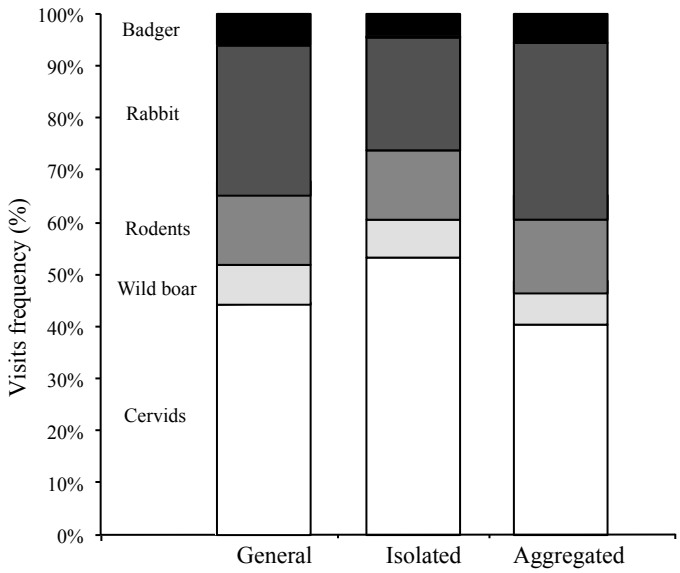

Figure 2. Percentage of visits for each frugivore in all *C. humilis* plants for the two years of the survey. The "General" bar is referred to all the dwarf palms without a spatial distribution classification.

Fruit harvesting

From 1672 fruits, 1220 (72.97%) were harvested by single frugivores: 47.59 % by cervids, 25.90% by rabbits, 15.06 % by rodents, 6.02 % by badgers and 5.42 % by wild boars. When we analyzed the fruits removed per visited infructescence we found that fruit harvesting differed among frugivores ($F_{4, 225}$= 7.78, P<0.0001). Although, we found low visitation and overall low fruit harvesting by badger, this had the highest probability of fruit harvesting per visit (0.40 ± 0.12), followed by wild boar (0.21 ± 0.08) and finally rodents (0.17 ± 0.06), cervids (0.16 ± 0.06) and rabbit (0.16 ± 0.06), which had similar fruit harvesting probability.

On the other hand, we found that the interaction between frugivore and aggregation level was significant ($F_{4, 225}$= 3.69, P=0.0063), indicating that at least one frugivore acted differently in aggregated and isolated plant. Through slice test, we found that some frugivores harvested fruit differently in isolated (test of slices; $F_{4, 225}$= 7.97, P<0.0001) and aggregated plants (test of slices; $F_{4, 225}$= 2.67, P=0.033). Badger had the highest fruit harvesting proportion in both the isolated and aggregated dwarf palms. Rodents and rabbits harvested more fruits in aggregated plants, and cervids and wild boar had no preferences (Figure 3).

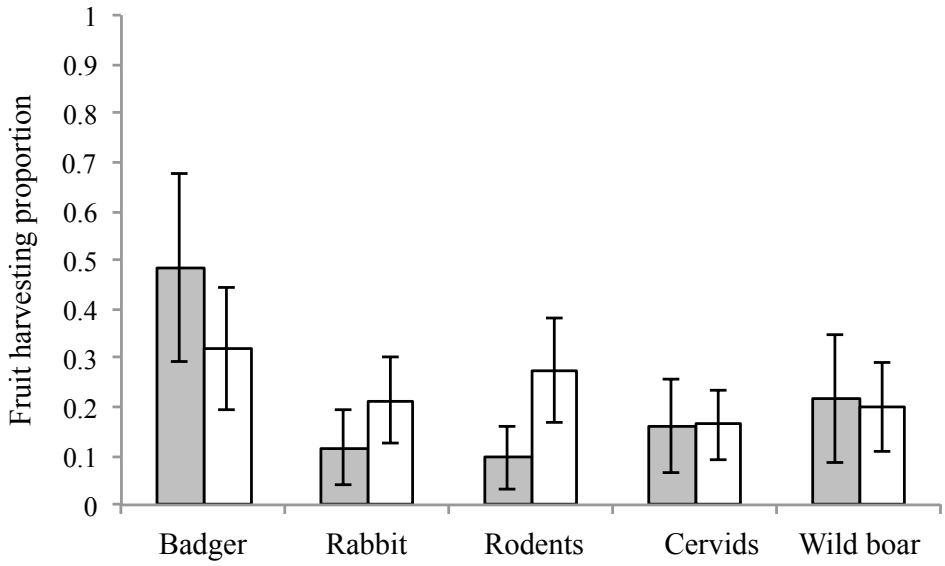

Figure 3. Fruit harvested proportion per visited dwarf palm for each frugivore in both isolated and aggregated female dwarf palms. The gray bars represent the isolated females and the white bars the aggregated. Error bars represent standard errors.

Finally, we combined the mean number of visited plant and fruit harvested proportion to obtain the fruit harvested effectiveness of each animal (Table1). Although cervids and rabbit showed low fruit harvesting in each visit, they "compensated" with high visit rates, reaching high effectiveness (Table 1). On the other hand, badger had the highest fruit harvested proportion but had the lowest visit rate, having relative medium values of fruit harvested effectiveness. Finally, the wild boar had the lowest values (Table 1).

Table 1. Frequency of visits of Eurasian badger, European rabbit, rodents, wild boar and cervids registered in 2012-2013 in Doñana National Park (SW Spain). Fruit harvested effectiveness is the product of the total frequency of visited plants and the proportion of fruit harvested per plant.

	Badger	**Rabbit**	**Rodents**	**Wild boar**	**Cervids**
Frequency of visits (%)	5.4	27.76	12.7	7.05	47.06
Fruit harvested proportion/plant	0.398	0.159	0.168	0.209	0.163
Fruit harvested effectiveness (%)	2.15	4.41	2.13	1.47	7.67

DISCUSSION

The first step to understand seed dispersal process in plants is to estimate the quantitative importance of different fruit consumers. A variety of factors determine this patterns, including plant aggregation level, frugivores visit frequency, frugivores behavior and number of seed handled (Schupp et al. 2010). Our study present the first attempt to quantify the *C. humilis* fruit removal patterns (visit frequency and fruit harvesting) by its frugivorous mammals assemblage. In particular, and contrary to our predictions, we found that spatial aggregation of fruiting plants had no effect on the rates of visit and fruit harvesting. However, we found differences between frugivores that respond differently to plants aggregation. Finally, accordingly with our prediction, we found that cervids (seed predator) and rabbits (pulp feeders) had the highest values of fruit harvesting effectiveness.

In terms of spatial distribution, the general trend is that aggregated plants are more attractive to frugivores due to higher fruit density (Sargent 1990, Carlo and Morales 2008), which in turn might increase the number of fruits removed per plant. However, most field studies of aggregation effects on rates of fruit removal or visitation by frugivores have documented competition or no

discernible effect of plant aggregations (Moore and Willson 1982; Saracco et al. 2005; Carlo and Morales 2008; Blendinger et al. 2008; Blendinger and Villegas 2011). We likewise did not find a discernible effect of the plants spatial context in the frugivory patterns. However, when we analyze the interaction of the spatial aggregation with each frugivore we found that they have different visits frequency and fruit harvesting. The more frequent visitors were the cervids followed by the rabbits, that are highly abundant in Doñana (Fedriani and Delibes 2009); instead less frequent visitors as badgers and foxes are quite scarce in Doñana (Fedriani and Delibes 2011).This is consistent with null models and previous empirical evidence suggesting that the frequency of fruit–frugivore interactions is highly dependent on frugivore abundance (Burns 2006). However, wild boar that is also abundant had very low visits frequency. Wild boar is able to harvest a lot of fruits (Matías et al. 2010), but the low visitation frequency and fruit harvesting showed that this animal had no special preference for the dwarf palm fruits.

In terms of fruit harvesting and plants aggregation level we found that pulp feeders (rodents and rabbits) appear to prefer aggregated dwarf palms over isolated ones, maybe because aggregations provide an excellent shelter, besides a good foraging resource to explode. Experiments in the field have demonstrated that shrub cover is valued by foraging rodents (Mattos and Orrock 2010, Malo et al. 2013), maybe because they can forage more efficiently in sheltered microsites (Fedriani and Manzaneda 2005). It in the case of rabbits they also prefer sites with more cover (Moreno et al. 1996). Furthermore, in poor vegetation areas they prefer plants aggregations for shelter and foraging (Moreno et al. 2004). The preference of pulp feeders for aggregated plants could reduce the fruits availability for badgers. This could explain why badger harvested more fruits in isolated plants, although the proportion was only slightly higher, corroborating partially our expectations. This negative interaction has been reported in various frugivore systems where pulp feeders reduced fruit availability for other frugivores (Tewksbury et al. 2008, Olesen et al. 2010).

Cervids and rabbits were the species that had the highest fruit harvesting effectiveness, harvesting overall more fruits than any other animal on the assemblage. These species consume many fruits of numerous fleshy-fruited plant species (e.g., Soriguer 1983, Santos et al. 1999, Dellafiore et al. 2007, Calviño-Cancela and Martín-Herrero 2009) and are very common in

Doñana. However, as ther are seed predators and pulp feeders of the dwarf palm fruits, their contribution to seed-dispersal is very poor, lessening the availability of fruits to legitimate seed disperser such as badgers. Thus, despite badger's medium effectiveness, they carry out almost all *C. humilis* seed-dispersal. Finally, in the case of fox its tracks always appeared with other frugivores and we were unable to dissect the harvested fruits by this species. Our results, corroborate the findings of Fedriani & Delibes (2011), where they show the dispersal effectiveness of the badgers.

Functional groups of frugivores severely affect the spatial patterning of seed dispersal (e.g. from highly scattered to highly aggregated) (Wiegand et al. 2007, Schupp et al. 2010, Fedriani and Wiegand 2014). In a previous work Jácome –Flores et al. found that the spatial distribution of the dwarf palm is characterized by the existence of superposed clustered patterns, with large clustered containing small ones and some individuals' randomly distributed. For instances, cervids destroyed the majority of the seeds, but occasionally dispersed seeds at short distances by spitting them out during mastication, leaving defleshed fruits near mother plants (authors personal observation). On the other hand, badgers were quite scarce visitors with overall medium fruit harvesting effectiveness, however, in each visit the seed harvested by this species was the highest. Badger has been considered a legitimate long distance seed disperser of dwarf palm fruits, with a 12-18% probability of seedling recruitment (Fedriani and Delibes 2011). Interestingly, though badgers act as long-distance dispersers (Fedriani et al. 1999, Revilla and Palomares 2002) they tend to defecate dwarf palm seeds in large latrines at relatively small separation (~10 m) from the neighborhood plants (Fedriani and Wiegand 2014), a fact that could increase the size of plants patches. Besides, these large clusters were overlaid by small-scale aggregations likely related to badgers feces that contain seeds strongly aggregated at small spatial scales (Fedriani and Delibes 2011). Finally, pulp removal by defleshers enhances germination and prevents microbial damage to seeds in endozoochorous plant species like the dwarf palm (Traveset et al. 2007, Fedriani and Delibes 2011), but this interaction fails to provide high-quality dispersal services to the dwarf palm. However, previous studies showed that dwarf palm seedlings, as in other plants (e.g. Montesinos et al., 2006; Roll et al., 1997; Wied and Galen, 1998), frequently grow and survive beneath conspecifics (Fedriani and Delibes 2011). Thus, rabbits and rodents increase short

distance dispersal creating small clustering patterns inside the large aggregations (e.g. Fedriani and Delibes 2013).

This study demonstrates that seed predators and pulp feeders were the functional guilds that handled more dwarf palm fruits. This could have demography consequences for the palm, reducing seed dispersal and eventually seedling establishment. For instance, the poor dispersal by small mammals like rodents and rabbits, would be changing the dispersal dynamics increasing the short-distance dispersal events over long distances, creating a more aggregated distribution. This has consequences over the seedling establishment, where seedlings could be more susceptible to predations near aggregated plants (Jácome-Flores et al., unpublished). Finally, the selective defaunation that our study are has been experiencing over the last decades made carnivores like badgers and foxes quite scarce, thus infrequent visitors with limited capacity to handled fruits. The disappearance of predators could increase the population size of rodents and rabbits and, therefore, the short distance dispersal events (Fedriani and Delibes 2013). Thus, the few fruits handled by badgers are essential for the dwarf palms long-distance dispersal in our study area, facilitating inter-patch connectivity and gene flow among dwarf palm populations (Jácome-Flores *et al.*, unpublished), as well as the slow colonization to new areas and population expansion.

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DISCUSIÓN GENERAL

Durante la segunda mitad del siglo XX el ecosistema del matorral mediterráneo ha experimentado fuertes cambios en los usos de suelo, fragmentación y reducción de hábitat y defaunación (Kosmas et al. 1997, Stoate et al. 2001, García-Ruiz 2010), con severas consecuencias sobre la supervivencia de las plantas que lo componen (Forget y Jansen 2007). Esto ha llevado a la reducción y extinción de muchas poblaciones vegetales, haciendo a estas últimas mas susceptibles a ciertos procesos denso-dependientes, limitando la supervivencia y el tamaño poblacional (Strong 1986, Hixon et al. 2002). El entendimiento de estos mecanismos de denso-dependencia y a qué escala espacial suceden es de vital importancia para la regulación y mantenimiento de muchas poblaciones de plantas y, por tanto, para el mantenimiento de la biodiversidad y el funcionamiento de los ecosistemas (Courchamp et al. 2008). Los efectos de la denso-dependencia espacial pueden variar en intensidad, signo, escala espacial y, además, suceder a lo largo de las diferentes etapas de la ontogenia de las plantas.

Esta tesis se ha centrado sobre el palmito *C. humilis*, una especie de palmera que, junto con *Phoenix theophrasti*, son las únicas palmeras nativas de Europa. El palmito es clave dentro del ecosistema mediterráneo, pues de él dependen varios procesos ecológicos; sin embargo, existe una rápida degradación y reducción del ambiente donde se distribuye, sobre todo en las costas de Italia, Francia y España. Esta tesis explora el efecto de la denso-dependencia sobre diferentes componentes del fitness del palmito (polinización, fructificación y dispersión de semillas) en dos poblaciones del área de Doñana (Martinazo y Matasgordas)- Ambas han sufrido recientemente un proceso de fragmentación y reducción (Fedriani et al. 2010). Para ello, nos hemos centrado en la cuantificación de los patrones espaciales y hemos analizado sus efectos sobre distintos componentes del fitness. Las localidades que escogimos para este trabajo fueron relativamente homogéneas en cuanto a hábitat, aunque han experimentado distintas presiones antropogénicas que han influido sobre las densidades poblacionales de la especie objeto de estudio.

Un resultado interesante de nuestro estudio fue la alta similitud de los patrones espaciales del palmito entre ambas poblaciones, a pesar de que una de ellas tiene una densidad poblacional cuatro veces mayor. Encontramos que los individuos de *C. humilis* presentan un patrón espacial

caracterizado por una agregación de los individuos a dos escalas: agrupaciones grandes con un diámetro entre los 38-44 m que están compuestas por pequeños grupos de plantas agrupadas a una distancia entre los 2.8-4 m. Asimismo, encontramos un patrón aleatorio superpuesto de manera independiente a las agrupaciones de plantas. También descubrimos que la distribución de sexos en estas agregaciones era totalmente aleatoria, es decir, que no existe ningún proceso de competencia intraespecífica o necesidades de microhábitat entre palmitos masculinos y femeninos que haga que alguno de los sexos se agregue de forma diferencial. Finalmente, en lo que a patrones espaciales se refiere encontramos que, al menos en una de las localidades, los individuos tenían tamaños similares dentro del radio de agregación, lo que podría significar que o bien no existe una competencia intraespecífica por los recursos que limite el crecimiento de los vecinos o hay algún proceso de facilitación.

Al relacionar estos resultados con las distintas estrategias de dispersión de los frugívoros que interactúan con el palmito encontramos que la dispersión podría ser la hipótesis que mejor podría explicar los patrones espaciales. Sin embargo, no descartamos que otros factores bióticos (competencia intra e interespecífica, herbivoría) y abióticos (sequias, incendios) hayan contribuido a la formación de su llamativo patrón espacial (Phillips y Macmahon 1981, Eccles et al. 1999, Tirado y Pugnaire 2003, Schurr et al. 2004, Fedriani et al. 2010).

El consumo de frutos y semillas del palmito es llevado a cabo por un diverso ensamble de mamíferos frugívoros formado por dispersores legítimos, despulpadores o dispersores ineficientes, y depredadores de semillas (Fedriani y Delibes 2011, Fedriani y Wiegand 2014). En concordancia con Schupp et al. (2010), observamos que lo que mas influye sobre la dispersión de semillas del palmito es la frecuencia de visitas de frugívoros, su abundancia local, y, por supuesto el número de semillas que manipulan. Encontramos que el palmito experimenta una seria limitación en la dispersión, puesto que los frugívoros más frecuentes y que más frutos consumieron fueron depredadores de semillas (ciervos) y dispersores ineficientes (conejos), mientras que los dos únicos dispersores legítimos (zorro y tejón) fueron escasos visitantes y consumieron un porcentaje muy bajo de frutos. Por tanto, la proporción de semillas que logran ser dispersadas lejos de la madre por dispersores legítimos es muy baja, mientras que la dispersión a corta distancia por los conejos parece ser la predominante. Esta situación aunada a la baja competencia intraespecífica

resulta en un alto reclutamiento de plántulas cerca de individuos adultos. A estas agregaciones se le suman las pocas semillas dispersadas por los tejones, quienes a pesar de actuar generalmente como dispersores a larga distancia (Fedriani et al. 1999, Revilla y Palomares 2002) tienden a defecar las semillas de palmito en grandes letrinas relativamente cercanas (~ 10 m) a otros individuos adultos (Fedriani y Wiegand 2014), hecho que podría explicar la formación de las dobles agregaciones encontradas. Finalmente, las semillas consumidas por el zorro tienden a ser defecadas de manera aleatoria (Fedriani et al. 2010), lo que contribuiría a la aparición de plantas distribuidas aleatoriamente.

Como ya establecimos en la introducción, los procesos de denso-dependencia espacial afectan a cómo la especie es utilizada como recurso por otras especies con las que interactúa (Ghazoul 2005, Aizen y Vázquez 2006, Muller-Landau et al. 2008, Fedriani y Delibes 2009, Wiegand et al. 2009). Existen diversos trabajos citados a lo largo de esta tesis que evidencian las distintas consecuencias de la agregación espacial y las interacciones planta-animal, específicamente en cómo afectan los patrones espaciales a los polinizadores (Aizen y Vázquez 2006, Nielsen et al. 2006), depredadores de semillas (Bonal et al. 2006, Muller-Landau et al. 2008, Wiegand et al. 2009, Fedriani et al. 2010) y dispersores de semillas (Malo y Suárez 1995, Silvius y Fragoso 2002, Fedriani y Delibes 2011). El palmito interactúa con varias especies de animales de forma mutualista (e. g. polinizadores y dispersores de semillas) y antagonista (depredadores de semillas y plántulas) (Fedriani et al. 2010, Fedriani y Wiegand 2014, Rodríguez et al. 2014), lo que la hace un buen modelo para evaluar los efectos de los patrones espaciales de las plantas sobre las relaciones planta-animal. Sin embargo, los patrones superpuestos de agregación espacial de esta planta tuvieron un efecto (o falta de él), cuando menos sorprendente sobre los polinizadores, el éxito de polinización y la dispersión de semillas.

El palmito mantiene una relación mutualista con el gorgojo denominada *nursery pollination,* en la cual la planta proporciona sitios (principalmente inflorescencias masculinas) para el desarrollo de las larvas del gorgojo *D. chamaeropsis* a cambio de servicios de polinización (Anstett 1999, Dufaÿ y Anstett 2003, Dufaÿ et al. 2004). Por tanto, el éxito de polinización en el palmito dependerá del número de polinizadores que las plantas puedan producir a costa de sus estructuras florales, lo que a su vez requiere de que polinizadores y flores se encuentren en el

tiempo y el espacio. Es por ello, que para estudiar la presencia y abundancia del gorgojos añadimos como variables explicativas el sexo de cada palmito, la sincronía de floración y la cantidad de flores, variables que en otros sistemas se ha visto que influyen sobre la presencia y abundancia de polinizadores (Eckhart 1991, Ashman 2005, Knight et al. 2005, Castilla et al. 2011). En general, se ha descrito que las larvas del gorgojo solo se pueden desarrollar en las inflorescencias de los palmitos masculinos (Dufaÿ y Anstett 2004), sin embargo, nosotros encontramos que al menos el 30% de las hembras tuvieron larvas en sus inflorescencias. Cabe mencionar que, en general, las inflorescencias femeninas con larvas fueron aquellas que tuvieron pocos frutos o ningún fruto, las cuales probablemente invierten poco para defenderse del gorgojo, permitiendo el desarrollo de sus larvas. Esto sugiere que los palmitos femeninos podrían sacrifican una fracción de sus inflorescencias para alimentar larvas de polinizadores, disminuyendo los costes del mutualismo y asegurando que los polinizadores sigan visitándolas. Asimismo, encontramos que el mutualismo palmito-gorgojo está condicionado por la sincronía de los ciclos ontogénicos de floración del palmito y emergencia de gorgojos, donde los palmitos más sincrónicos tendrán más larvas que los asincrónicos. Además, los palmitos experimentan una limitación en la cantidad de polinizadores, puesto que la presencia de larvas en los palmitos disminuyó con la cantidad de inflorescencias, perdiendo un recurso de ovoposición por falta de polinizadores y limitando la polinización (ver abajo).

A nivel poblacional observamos que la distribución espacial del palmito no influye sobre los patrones espaciales de presencia y abundancia de larvas del gorgojo. Esto se debe a que las plantas están agregadas con un radio menor a la distancia máxima a la cual el gorgojo se puede mover (al menos 225 m), por lo que todas las plantas con valores altos de sincronía y pocas inflorescencias tendrán una gran probabilidad de tener larvas sin importar su ubicación espacial. Esto tuvo un efecto sobre el éxito de polinización temprana, ya que observamos que el nivel de agregación de las plantas no tuvo efecto sobre la producción de frutos. Es decir, que ninguna de las plantas que consideramos aisladas en las parcelas observacionales y experimentales estuvo lo suficientemente lejos como para notar algún efecto de limitación en la polinización por distancia.

En sistemas con niveles altos de agregación las plantas están situadas a distancias muy cortas, lo que facilita el transporte de polen entre individuos e incrementa la producción de frutos

tempranos (Klinkhamer et al. 1989, Robertson y MacNair 1995, Gascoigne et al. 2009, Fedriani et al. 2015). Sin embargo, a nivel poblacional encontramos que la densidad de individuos florales tuvo un efecto negativo sobre el éxito de polinización, de forma que la baja densidad de individuos florales en Martinazo incrementó el éxito de polinización. Esto puede ser debido a un aumento de la competencia intraespecífica en poblaciones con altas densidades (Harper 1977, Padien y Lajtha 1992, Tilman 1994, Vilà y Sardans 1999, Mustajärvi et al. 2001, Craine 2005). Los palmitos en poblaciones con alta densidad podrían experimentar el efecto directo de la competencia por los recursos, tales como la luz, el agua y los nutrientes (Metcalfe y Kunin 2005, Gunton y Kunin 2009). Esto podría explicar por qué las plantas en Martinazo son capaces de ofrecer una mayor cantidad de recursos florales. Asimismo, las plantas en poblaciones con bajas densidades experimentan menos competencia por los servicios de polinización; al haber menos plantas con inflorescencias, disminuyó la competencia intraespecífica por los pocos polinizadores y se incrementó el éxito de polinización temprano. Esto concuerda con los resultados de Jakobsson et al. (2009) y Otway et al. (2005), quienes encontraron que la densidad local de flores tenía un efecto negativo sobre el éxito de polinización, ya que la probabilidad de visitas de los polinizadores decrecía en altas densidades de floración. Sin embargo, los experimentos de polinización manual revelaron que el posible efecto limitante de la cantidad de polen es muy pequeño, dejando a la competencia por nutrientes y la asignación de recursos como las variables que mejor explican nuestros resultados de éxito de polinización.

Por supuesto el éxito de polinización y el desarrollo de los frutos está condicionado por la estructura genética espacial (EGE) de las plantas, creada cuando los individuos espacialmente cercanos lo son también genéticamente (Vekemans y Hardy 2004). Sin embargo, a pesar de la alta agregación espacial de los palmitos, el análisis genético evidenció que los individuos son genéticamente distintos en ambas poblaciones y que no existe una EGE, sino que los acervos genéticos muestran una distribución aleatoria. Ello hace pensar que no debería esperarse que el aporte de polen procedente de individuos cercanos cause efectos genéticos deletéreos en la fructificación (e. g. Fedriani et al. 2015). Esto lo corroboramos mediante los resultados del número de frutos desarrollados, en los que encontramos un alto porcentaje de frutos desarrollados sin importar el grado de agregación en las plantas.

Finalmente, mediante los experimentos de polinización fuimos capaces de medir la contribución de la polinización anemófila, ampliamente sugerida y nunca cuantificada para el palmito (Herrera 1989, Anstett 1999, Dufaÿ y Anstett 2004). Encontramos que el aporte de polen transportado sólo por el viento fue inferior al 0.1 %, por lo que la polinización del palmito la lleva a cabo casi exclusivamente el gorgojo *D. chamaeropsis*. Asimismo, encontramos que a pesar de que la inclusión manual de polen debería de haber polinizado la mayoría de los óvulos, el resultado siempre fue el mismo: menos del 30% de los óvulos logró convertirse en fruto. Estos resultados fueron muy similares a los de la polinización manual, lo que nos hace pensar que de las tres drupas potenciales por flor normalmente sólo se desarrolla una, lo que sugiere que puedan existir efectos de aborto selectivo en el que se selecciona el fruto con "genotipo superior" (Stephenson 1981, Kozlowski y Stearns 1989) y/o competencia entre drupas hermanas por los recursos (Mock y Parker 1997)

En otro orden de ideas, encontramos que la frugivoría en general no se ve afectada por los patrones espaciales de las plantas, pues sin importar el nivel de agregación las tasas de consumo de frutos fueron similares. Sin embargo, cuando analizamos la interacción de la agregación espacial para cada frugívoro encontramos que tienen diferente frecuencia de visitas de frecuencia y consumo de frutos. Encontramos que los visitantes más frecuentes fueron los cérvidos, seguidos de los conejos, ambos muy abundantes en Doñana (Fedriani y Delibes 2009). Sin embargo, estas dos especies son dispersantes ineficaces del palmito, por lo que los frutos consumidos por ellos limitan los procesos de dispersión reduciendo la posibilidad de que los frutos sean ingeridos por dispersores legítimos como el tejón.

En términos de consumo de frutos en relación con el nivel de agregación de las plantas, los despulpadores (conejos y roedores) consumieron más frutos en las plantas agregadas. Este efecto de denso dependencia positiva se explica por el comportamiento de estas especies, que prefieren zonas de mayor densidad de vegetación no sólo porque ofrecen mayor cantidad de comida (frutos), sino también porque la cobertura vegetal les provee de un excelente refugio contra los depredadores (Moreno et al. 2004, Fedriani y Manzaneda 2005). La preferencia por las plantas agregadas pudo reducir el número de frutos disponibles para los dispersores legítimos (e. g. tejones). Esta interacción negativa ha sido reportada en diversos sistemas de frugivoría (Fedriani y

Delibes 2013). Quizás por ello los tejones consumieron más frutos en plantas poco agregadas, en las que hubo mayor disponibilidad de frutos.

Lo anterior podría tener consecuencias sobre el reclutamiento de plántulas y los patrones espaciales del palmito, ya que al aumentar los eventos de dispersión a corta distancia se genera una distribución de semillas y plántulas altamente agregada. Fedriani y Delibes (2011) y Rodríguez et al. (2014) demostraron que las semillas de palmito debajo de las plantas madre experimentaban mayor depredación por escarabajos y ataques de patógenos. Asimismo, observamos un efecto de denso-dependencia positiva en la supervivencia de plántulas, ya que la depredación de plántulas fue mayor en las plantas más agregadas (datos no incluidos).

En resumen, los resultados de esta tesis indican que la agregación espacial de los palmitos influye de manera distinta sobre las distintas interacciones mutualistas y antagonistas. Para entender el efecto neto de la denso-dependencia espacial hay que diseccionar cada uno de los componentes del ciclo reproductivo de la planta. En el caso de la polinización, los resultados están influenciados por la distribución espacial y abundancia de los gorgojos. Gunton y Kunin (2009) estipulan que los efectos directos de la denso-dependencia sobre la atracción de polinizadores y herbívoros sólo son visibles a escala poblacional y no individual. Por ello, a pesar de que a nivel individual las plantas se encuentran limitadas por la cantidad de polinizadores, sólo a nivel poblacional encontramos un efecto de denso-dependencia negativa, donde las plantas en el sitio con mayor densidad parecen competir por los servicios de polinización. Esta relación se ve reforzada por el alto éxito de fructificación tardía que tuvo la población con menor densidad de plantas. En términos generales encontramos que la frugivoría no se veía afectada por los patrones espaciales del palmito. Esto se puede explicar por el gran porcentaje de frutos que consumieron los depredadores de semillas, quienes no mostraron preferencia por plantas agregadas o poco agregadas. Sin embargo, en términos de dispersión de estos frutos, encontramos un efecto de denso-dependencia negativa a nivel de planta pero no a nivel poblacional, pues los dispersores legítimos “preferían” las plantas poco agregadas. Por otro lado, los conejos y roedores en conjunto dispersan más frutos que los dispersores legítimos y la dispersión a cortas distancias esta relacionada positivamente con la densidad de plantas. Finalmente, este efecto se neutraliza debido a la gran depredación de plántulas cercanas a plantas agregadas.

Cualquier alteración en los patrones espaciales y en la densidad de plantas por efectos de la fragmentación se traducirá en una alteración de la presencia y abundancia de las especies animales con las que interactúa. En el caso de la polinización, la reducción y el aislamiento de las poblaciones de plantas reduciría los sitios de reproducción de los gorgojos disminuyendo también sus poblaciones Ello puede reducir en gran medida el intercambio genético a través de polen (Richards et al. 1999), causando a largo plazo una depresión endogámica, una reducción del fitness de las plantas y por último una disminución en la viabilidad general de la población (Gilpin y Soulé 1986, Willi et al. 2005).

En nuestras áreas de estudio la defaunación selectiva y la introducción de ganado son una de las presiones antropogénicas que más ha influido sobre el éxito reproductivo del palmito. En la últimas décadas del siglo pasado las actividades de caza redujeron drásticamente las poblaciones de varios mamíferos como los tejones y zorros, dispersores de gran variedad de semillas (e. g. palmito, camarina, sabina, enebro marítimo, zarzamora, olivilla), limitando los servicios de dispersión a larga distancia, la colonización de nuevas áreas y la regeneración de poblaciones. Asimismo, la disminución de las poblaciones de estos dispersores influye en el incremento de las poblaciones de roedores y conejos, quienes son dispersores ineficientes pues abandonan las semillas debajo de la planta madre, donde el reclutamiento y supervivencia de plántulas es bajo. La conservación de los dispersores legítimos de semillas es necesaria para el mantenimiento y, en su caso, expansión de esta especie y del matorral mediterráneo en general (Suárez-Esteban et al. 2013), por lo que el mantenimiento de las especies de plantas de las que depende y los servicios ecosistémicos que proveen estas especies deben de ser considerados a la hora de manejar las poblaciones de éstos mamíferos (Schröter et al. 2005). Por otro lado, la presencia de ganado junto con la de los herbívoros silvestres (*C. elaphus, S. scrofa, O. cuniculus*) ha incrementado la presión de herbivoría sobre las plántulas, impidiendo o ralentizando también la expansión y regeneración de las poblaciones de palmito. Si se pretende recuperar las poblaciones del palmito o usarlo como especie pionera para la regeneración de hábitats degradados, hay que considerar la exclusión del ganado.

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CONCLUSIONES

1. El palmito *C. humilis* presenta un patrón espacial caracterizado por una agregación de los individuos a dos escalas. Existen agrupaciones grandes con un diámetro entre los 38-44 m, compuestas por pequeños grupos de plantas agrupadas a una distancia entre los 2.8-4 m. Asimismo, encontramos un patrón aleatorio sobreimpuesto de manera independiente a las agrupaciones. Esta distribución coincide con los patrones de dispersión de los dispersores de semillas de palmito como el tejón, que dispersa a largas distancias y de forma agregada; los zorros también dispersan las semillas a larga distancia pero de forma menos agregada; y los conejos dispersan las semillas debajo o muy cerca de la planta madre.

2. No existe agregación espacial de los sexos. Esta distribución le confiere al sistema grandes ventajas para la polinización, ya que las plantas tuvieron mayor probabilidad de que los polinizadores encuentren y transporten el polen entre los individuos. Esto fue confirmado mediante los experimentos de polinización en los que, aunque éxito de cuajado natural fue bajo, resultó muy similar al de la polinización manual.

3. Los individuos mostraron tamaños similares dentro del radio de agregación, lo que podría significar que existe una baja competencia intraespecífica por los recursos entre individuos de la misma cohorte.

4. Los palmitos machos tienen mayor presencia y abundancia de larvas de *D. chamaeropsis* aunque, en contra de lo descrito en la bibliografía, encontramos que las hembras también pueden albergar una cantidad considerable de larvas, lo que implica el "sacrificio" de sus inflorescencias con pocos frutos y el ofrecimiento de inflorescencias no polinizadas como sitios de puesta. Esto sugiere que los gorgojos son recompensados por sus servicios de polinización también por las plantas hembra mediante sitios donde desarrollar sus larvas, pagando el coste del mutualismo.

El nivel de agregación del palmito no afecta a los patrones espaciales de presencia y abundancia de larvas del gorgojo. Sin embargo, estas dos variables fueron afectadas por la sincronía de floración de cada individuo con respecto a los demás y la cantidad de inflorescencias

que produce. Los palmitos sincrónicos y con pocas inflorescencias tuvieron mayor probabilidad de albergar larvas.

5. En general, el nivel de agregación del palmito no tuvo un efecto observable sobre el éxito de polinización. No obstante, notamos que a nivel poblacional el éxito de fructificación temprano de frutos fue menor en la población con mayor densidad de individuos. Esto podría relacionarse con el hecho de que el palmito tiene un sistema de polinización especializado, en el cual existe una alta competencia intraespecífica de las plantas por los servicios de polinización limitando la cantidad de polen que las plantas reciben.

6. A pesar de que los palmitos están fuertemente agregados, los individuos de una misma agregación fueron genéticamente distintos entre sí en ambas poblaciones, por lo que no es esperable un efecto de depresión endogámica. Ello es coherente con el alto éxito de fructificación tardía detectado. La falta de estructura espacial de los genotipos es compatible con: 1) la alta movilidad de los gorgojos, capaces de transportar polen de plantas muy lejanas, y 2) los distintos eventos de dispersión a larga distancia por zorros y tejones.

7. La dispersión de semillas tampoco parece haber sido afectada por la distribución espacial de los palmitos. Sin embargo, detectamos cierta limitación en la dispersión, donde un gran porcentaje de los frutos fueron depredados por ungulados y otro porcentaje dispersado ineficientemente por conejos y roedores. A su vez, las altas tasas de consumo de frutos de estos animales limitaron considerablemente el número de frutos disponibles para los dispersores legítimos (tejón y zorro).

8. Durante las últimas décadas nuestras áreas de estudio han experimentado defaunación selectiva, que ha reducido la abundancia de carnívoros como tejones y zorros. Este hecho ha disminuido su abundancia y con ella su capacidad para consumir frutos. Precisamente por ello, los pocos frutos de palmito que consumen estas especies son especialmente importantes para la dispersión a larga distancia, facilitando la conectividad entre parches, el flujo de genes entre las poblaciones de palmito y la lenta colonización nuevas áreas.

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